REMARKS

I. Status of the Claims

Claims 98-122 are pending. Claims 118-122 have been withdrawn from consideration. Claims 98-117 have been rejected.

II.   Restriction Requirement

The applicants continue to consider that the restriction requirement made in the Office Action Mailed August 30, 2007 was improper for the reasons given previously. A petition requesting reconsideration of the restriction requirement is being filed herewith.

III.   Response to Claim Rejections

A. Rejection of Claims 98-117 under 35 U.S.C. § 112, First Paragraph (Enablement).

Claims 98-117 were rejected under the enablement requirement of 35 U.S.C. § 112, first paragraph. The applicants respectfully traverse the rejection.

The Office Action contends that the specification does not reasonably provide enablement for a solvate or a hydrate of a compound of formula (A). The Office Action states:

> It has been estimated that approximately one-third of the pharmaceutically active substances are capable of forming crystalline hydrates. Predicting the formation of solvates or hydrates of a compound and the number of molecules of water or solvent incorporated into the crystal lattice of a compound is complex and difficult. Each solid compound responds uniquely to the possible formation of solvates or hydrates and hence generalizations cannot be made for a series of related compound (See Vippagunta, et al.)

> The scope of "solvate" is not adequately enabled or defined. Applicants provide no guidance as how the compounds are made more active in vivo. Solvates and hydrates cannot always be predicted and therefore are not capable of being claimed if the applicant cannot properly enable a particular hydrate or solvate.

Applicants enjoy *a presumption* that the specification, which discloses how to make and use the claimed invention, complies with the first paragraph of 35 U.S.C. § 112, unless there is a reason to doubt the objective truth of the specification. MPEP 2164.04 (citing *In re Marzocchi,*

439 F.2d 220, 224 (C.C.P.A. 1971)). The initial burden of establishing a basis for denying patentability to a claimed invention therefore rests upon the Office. *Id.* "It is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning *which is inconsistent with* the contested statement." MPEP 2164.04 (citing *In re Marzocchi*, 439 F.2d 220, 224 (C.C.P.A. 1971))(emphasis added).

An application satisfies the enablement requirement if the disclosure has sufficient information to enable the person skilled in the pertinent art to make and use the claimed invention without undue experimentation. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). The test for whether experimentation would be undue is not merely quantitative since a considerable amount of experimentation is permissible, if it is merely routine. *Id* at 737. The fact that experimentation may be required and may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), *aff'd. on other grounds sub nom., Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985). *See also In re Wands*, 858 F.2d at 737. The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976).

It appears from the reasons given for the rejection that the Examiner contends that formation of crystalline solvates is somewhat unpredictable, particularly with regard to the number of molecules of water or solvent incorporated into the crystal lattice of a compound, and whether the compounds are more active *in vivo*. The Office Action states that since "[s]olvates and hydrates cannot always be predicted [they] are not capable of being claimed if the applicant cannot properly enable a particular hydrate or solvate."

The applicants respectfully point out that absolute predictability is not required in order to satisfy the enablement requirement. Further, the claims do not recite having a particular number of solvent atoms, or a particular structural lattice, or solvates which are more active in vivo, which seem to be the issues addressed by Vippagunta. The applicants' claims, in fact, do

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not recite any particular features of hydrates and solvates that they encompass. Rather, the claims include all forms of the compounds defined in claim 1, including any hydrate or solvate. Even if solvate formation were somewhat unpredictable, as the Examiner contends, the claims would still satisfy the enablement requirement because such experimentation as might be required to prepare salts or hydrates of the compounds of the invention would be routine and well within the capacity of the skilled artisan, and would therefore not be undue, as is demonstrated by the references cited below.

The Office Action couches its discussion of issue of enablement in terms of the factors considered in *Wands*. It would therefore be instructive to compare the complex, unpredictable antibody technology described in *Wands* with the simple problem of making solvates and hydrates which the Office makes an issue in the present application.

The issue in *Wands* was whether the patentee had adequately enabled one skilled in the art to make certain high-affinity IgM antibodies. *Wands*, 858 F.2d at 735. The PTO had rejected the claims, stating that the production antibodies was unpredictable and unreliable, thus requiring undue experimentation. *Id.* However, the Federal Circuit reversed, finding the claims to be enabled as a matter of law. The court made the point that even though the screening required to produce the antibodies was labor-intensive with a lot of steps (e.g., immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells, cloning the hybridoma, screening the resulting antibodies, etc.), all the methods needed to practice the invention were well known, and the amount of effort was not excessive enough to be undue *despite any unpredictability* associated with making antibodies. *Id* at 740.

In stark contrast to the complex and unpredictable antibody-making procedures at issue in *Wands*, the preparation of hydrates and solvates of a given organic molecule is substantially easier, overwhelmingly simpler, requires significantly fewer steps, and demands much less time than for the preparation of a monoclonal antibody. Accordingly, since the court concluded that the preparation of a monoclonal antibody was enabled *as a matter of law* despite the complex and lengthy process involved, it is unreasonable for the patent office to reject hydrates and solvates as lacking enablement given that they are infinitely simpler to make. The table below

provides a step-by-step comparison of some of the major steps involved in the production of a monoclonal antibody (as disclosed in *In re Wands*) and the one step involved in making a hydrate or solvate. The experimentation involved in the production of a monoclonal antibody is tremendously more complex and time-consuming than forming a solvate, yet the court concluded that it was not excessive and undue.

Step	Monoclonal Antibody	Solvate or hydrate
1	immunize animal	Expose the compound to solvent or water
2	remove the spleen from the immunized animal	
3	separate the lymphocytes from the other spleen cells	
4	mix the lymphocytes with myeloma cells	
5	treat the mixture to cause fusion between the lymphocytes and the myeloma cells to make hybridomas that hopefully secrete the desired antibody	
6	separate the hybridoma cells from the unfused lymphocytes and myeloma cells by culturing in a medium in which only hybridoma cells survive	
7	culture single hybridoma cells (often 100 of different cells) in separate chambers	
8	assay the antibody secreted from each hybridoma culture to determine if it binds to the antigen	
Total Time	Months	About 1-2 days

Thus, to say the rejection of the claims based upon an assertion that the preparation of solvates would require "undue" experimentation is clearly inconsistent with the Federal Circuit's holding finding that the claims to forming antibodies were enabled as a matter of law in *Wands*.

Although making monoclonal antibodies involves a greater amount and complexity of experimentation than is involved in forming solvates, the preparations of monoclonal antibodies and solvates share the characteristic that the step(s) involved are well known and routine.

Applicants provide herewith evidence that solvate is easy, simple, requires few steps, and demands little time, and that the person of skill in the art routinely engages in such experimentation, and that the techniques for performing such experimentation are well known.

To make hydrates and solvates, samples of the organic compound are simply exposed to water or various different solvents. Exposure of the organic compounds to water and various solvents is conducted through simple and routine methods such as letting the samples sit open to air for set amounts of time, as well as slurrying and/or crystallizing the samples from water or solvent. In fact, it is difficult to conceive of a scientific method that is simpler to perform than placing a powder on a dish and letting it sit out on a humid day. Other typical procedures for making and identifying hydrates and solvates are described on pages 202-209 of K.J. Guillory, "Generation of Polymorphs, Hydrates, Solvates, and Amorphous Solids," in: Polymorphism in Pharmaceutical Solids, ed. Harry G. Brittan, Vol. 95, Marcel Dekker, Inc., New York, 1999, a copy of which is provided herewith.

Once solvates are formed, they can be readily analyzed by routine methods. Examples of such techniques include thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Karl Fischer titrimetry, X-ray diffractions (single crystal or powder), infrared spectroscopy (IR), polarized light microscopy, and hot stage microscopy or other routine techniques to detect and quantify the presence of solvate molecules in the sample. As evidence thereof, see page 18, right column, Vippagunta et al., which has been cited by the Office.

While there may be many solvents and conditions to try, the screen merely uses methods that are very well known in the art and considered quite simple. In fact, the process is *so* routine as to be amenable to high throughput screening, for example high throughput crystallization as described, for example, in Morisette, *et al.*, *Adv. Drug Delivery Rev.*, **2004**, *56*, 275-300, a copy of which is provided herewith.

The Office Action attempts to base its enablement rejection solely on the alleged unpredictability of solvate formation and the fact that no specific examples of solvates have been described in the specification. *Wands* establishes that unpredictability (which was the main grounds of improper enablement rejection in *Wands*), even if it were established, is not dispositive. Also, there is no requirement for a "working" example if the disclosure is such that one skilled in the art can practice the claimed invention. *In re Borkowski*, 164 U.S.P.Q. 642 (C.C.P.A. 1970); *Ex parte Nardi*, 229 U.S.P.Q. 79 (Pat. Off. Bd. App. 1986). Given that one skilled in the art could make and identify various hydrates and solvates of a particular organic molecule using the routine screening methods discussed above, no working example is necessary to enable the invention. *Wands*, in fact, mandated that numerous factors be considered in evaluating enablement rather than the narrow approach taken by the Office here.

It is respectfully submitted that any unpredictability or the absence of examples of solvates specifically described as solvates or hydrates should be found to be clearly outweighed by the other factors considered in *Wands*.

As to the **nature of the invention**, the application is directed to pharmaceutical compounds and salts thereof. It is well known that stable, crystalline solvates and hydrates can be formed from such compounds (even though the claims do not require the solvates and hydrates to be crystalline or stable).

As to **state of the prior art**, and **predictability in the art**, the Office points out that whether a hydrate or solvate will be formed in a given case, and its exact structure, cannot be reliably predicted *a priori*. Although the Office focuses on this supposed unpredictability, the Office acknowledges that the prior art shows that a high percentage of pharmaceutically active compounds are found to be capable of forming crystalline solvates. Thus, it must be acknowledged that while predicting whether a given solvate will form might be unpredictable, formation of solvates generally is not at all unusual, and can be performed using routine and predictable methods. The Office does not acknowledge the well-established and routine methods established in the art for preparing, screening, and evaluating solvates and hydrates.

As to the **amount of direction**, and the presence or absence of **working examples**, the applicants respectfully point out the absence of specific direction or working examples is not required when the techniques required to practice the invention are entirely routine and well known. As to the aspect of the invention at issue here (formation of solvates and hydrates), the techniques for preparing, screening, and evaluating solvates and hydrates are well known and routine, and nothing would be gained by describing such methods in the specification. A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991).

Although the Office recognizes that the **level of skill in the art** is high, the Office does not accord sufficient weight to this factor in considering enablement of the claims. The person skilled in the art who would prepare solvates or hydrates of the compounds of the invention would typically be a highly skilled artisan, such as a Ph.D. qualified scientist, in the art of chemistry or pharmaceutical formulation. The person skilled in the art would be familiar with the routine techniques available for preparing, screening, and evaluating solvates and hydrates. The person skilled in the art would be capable, if necessary, of routinely screening many different compounds of the formulae defined in the claims, using a variety of solvents, and conditions for solvate or hydrate formation, and would have routine methods for evaluating the results of such screening.

Based on the foregoing it is clear that the **quantity of experimentation** needed to practice the invention would not be undue. Insofar as there might be unpredictability in solvate formation, the art has responded by providing routine, high throughput methods for preparing, screening, and evaluating solvates and hydrates. *Wands* has acknowledged that routine screening does not constitute undue experimentation.

The applicants note that even a cursory search of the U.S.P.T.O. database of issued patents suggests a substantial number of pharmaceutical patents with claims referencing solvates and hydrates, yet having no enablement rejections to the same: see, e.g. Patents. Nos. 7,232,823, 7,230,024, 7,230,002, 7,229,991, 7,227,027, 7,211,591, 7,173,037, 7,157,466, and 7,105,523.

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The applicants see no difference between these patents and the present application with respect to enablement of hydrates and solvates.

Since the preparation of solvates is the type of experimentation that is routinely engaged in the art, and merely involves the use of well known methods without excessive effort, applicants respectfully request that the rejection of claims 98-117 under the enablement requirement of 35 U.S.C. § 112 first paragraph based upon the recitation of solvates be withdrawn.

B. Rejection of Claims 99 and 112-115 under 35 U.S.C. § 112, Second Paragraph.

Claims 99 and 112-115 were rejected as indefinite under 35 U.S.C. § 112, second paragraph. The applicants traverse the rejection.

The Office Action states that:

> The phrase "having formula" renders the products indefinite as the phrase "having formula" can be considered open ended language when not clearly defined and therefore is including additional subject matter in the compounds of the formula A that is not described in the instant specification and is not particularly pointed out or distinctly claimed. A claim to a chemical compound cannot be open-ended, but must be claimed with precision.

The Examiner suggests that the rejection could be overcome by amending the phrase "having formula" to read "of structure".

The applicants respectfully disagree that the use of the phrase "having formula" renders the rejected claims fatally indefinite, or that an amendment to the claims is required in order to make the claims clear. MPEP 2173.02 explains:

> The examiner's focus during examination of claims for compliance with the requirement for definiteness of 35 U.S.C. 112, second paragraph, is whether the claim meets the threshold requirements of clarity and precision, not whether more suitable language or modes of expression are available. ...

> The essential inquiry pertaining to this requirement is whether the claims set out and circumscribe a particular subject matter with a reasonable degree of clarity

and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:

    (A) The content of the particular application disclosure;

    (B) The teachings of the prior art; and

    (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. ...

If the language of the claim is such that a person of ordinary skill in the art could not interpret the metes and bounds of the claim so as to understand how to avoid infringement, a rejection of the claim under 35 U.S.C. 112, second paragraph, would be appropriate. However, if the language used by applicant satisfies the statutory requirements of 35 U.S.C. 112, second paragraph, but the examiner merely wants the applicant to improve the clarity or precision of the language used, the claim must not be rejected under 35 U.S.C. 112, second paragraph...

The applicants respectfully submit that the Office has not provided reasoning adequately explaining how the phrase "having formula" would render it impossible for the person skilled in the art to interpret the metes and bounds of claims 99 and 112-115.

Although the Office Action states that the phrase "having formula" is open ended, the Examiner has not explained *how* any ambiguity arises as to what the claims in question do, or do not cover when the Examiner does not appear to contend that the formulae defined in these claims are ambiguous. Although the Examiner seems to consider that the claims somehow provide for additional subject matter, the Examiner has not explained *what* additional subject matter could be impermissibly included that could justify rejecting the claims as vague and ambiguous.

The applicants respectfully point out that, in general, there is nothing impermissible about using open-ended language in claims, and that claims are not generally required to exclude additional, unrecited elements. In fact, the use of open-ended language is expressly sanctioned in the MPEP. *See generally* MPEP 2111.03 (explaining that "[t]he transitional term "comprising" ... is inclusive or open-ended and does not exclude additional, unrecited elements or method steps."). The applicants respectfully point out that the term "having" used in the context of claim language is not necessarily considered to be open-ended. Therefore, the

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meaning of such a term must be considered in the context in which it is used. MPEP 2111.03 points out:

> Transitional phrases such as "having" must be interpreted in light of the specification to determine whether open or closed claim language is intended. *See, e.g., Lampi Corp. v. American Power Products Inc.*, 228 F.3d 1365, 1376, 56 USPQ2d 1445, 1453 (Fed. Cir. 2000) (The term "having" was interpreted as open terminology, allowing the inclusion of other components in addition to those recited); *Crystal Semiconductor Corp. v. TriTech Microelectronics Int'l Inc.*, 246 F.3d 1336, 1348, 57 USPQ2d 1953, 1959 (Fed. Cir. 2001) (term "having" in transitional phrase "does not create a presumption that the body of the claim is open"); *Regents of the Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1573, 43 USPQ2d 1398, 1410 (Fed. Cir. 1997) (In the context of a cDNA having a sequence coding for human PI, the term "having" still permitted inclusion of other moieties).

There is nothing unclear about the use of the word "having" in claims 99 and 112-115.

It is perfectly conventional, both in everyday language and the language of chemistry, to associate the properties of an object by referring to the object as "having" particular properties. One might refer to a house, for example, as "having" a particular floor plan. A room may be said to "have" a particular temperature. Chemists use the verb "to have" to associate a compound with its properties in the same, conventional, sense. It is therefore perfectly conventional to refer to a compound as "having" a particular structural formula, meaning that the structural formula represents the structure of the molecules of the compound. The structural formula that the compound is said to "have" represents how the atoms are connected together (as a floor plan represents how the rooms of a house are connected together).

Here, claims 99 and 112-115, which refer to a compound as "having" the particular formulae defined therein, in the same conventional sense as described above, are perfectly clear and unambiguous. There is nothing ambiguous about stating that a compound "has" a particular structure when the structure itself is clear. Here, the Examiner has not alleged that the formulae in claims 99 and 112-115, are unclear, but only objects to the use of the word "having".

The only reasoning provided by the Examiner to support the rejection is the contention that the word "having" is somehow "open ended", such that it includes "additional subject

matter", and that this is somehow not permitted when claiming chemical compounds because "a claim to a chemical compound cannot be open-ended, but must be claimed with precision."

The applicants disagree with the Examiner's reasoning.

Claims 99 and 112-115 are perfectly clear because the phrase "having formula" merely means that the compounds defined by those claims must have a structure within the definition of the formula provided in each of those claims. The claims are clear and unambiguous because the definition of the formula is clear and unambiguous. There is nothing open-ended in the definitions of the formulae for each of these claims. As such, the use of the word "having" does not create any ambiguity. The compounds are therefore defined "with precision" as the Examiner would desire.

As to the Examiner's suggestion that the use of the word "having" in claims 99 and 112-115 somehow causes the claims to be impermissibly open-ended, if it is the Examiner's contention that the claims are open-ended insofar as the structure of the compound which is being claimed, then the applicants disagree. The structure of the compound being claimed is clear and unambiguous because the definitions of the formulae in the claims are clear and unambiguous.

If the Examiner's contention is that the claims might be considered open-ended in some other respects, the applicants would point out that the claims are not open-ended in any way which creates ambiguity or lack of clarity. The claims might be open-ended in the sense that they do not require that the compounds be pure, and therefore do not exclude the presence of additional, unrecited components which might be present together with the compound under some circumstances. For example, the claims are intended to cover solutions of the compound in water or other solvents, pharmaceutical compositions containing the compound along with one or more pharmaceutical excipients or carriers, or water or solvent present in a solid phase form of the compound. However, this is not impermissible, nor does it create any ambiguity in what is claimed.

Based on the foregoing, the applicants respectfully submit that claims 99 and 112-115 are not indefinite, and that the rejection should therefore be withdrawn. Although the applicants

have considered the Examiner's suggestion of amending the claims by amending the phrase "having formula" to read "of structure", the applicants believe that the clarity and precision of the claims with the proposed amendment would depend, like the present claims, solely on the clarity of the definitions of the formulae. Since the clarity of the formulae in claims 99 and 112-115 is not disputed, the applicants respectfully request that the rejection be withdrawn.

IV.   Response to Claim Objections

Claims 98-117 were objected to as containing non-elected subject matter and the Examiner suggests that the applicant should amend the claims to delete the non-elected subject matter in order to overcome the objection. The applicants respectfully traverse this objection. The applicants believe the requirement is improper because the restriction requirement is improper. Moreover, the requirement is improper because the claims in question are elected.

V.   Conclusion
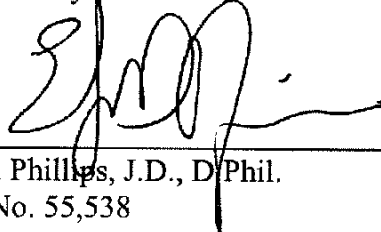
Based on the foregoing, the applicants believe that all the objections and rejections in the Office Action have been addressed and that, as such, the claims are in condition for allowance. An early action toward that end is therefore earnestly solicited.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing Attorney's Docket No.: 20750-034US1 / 004.US3.PCT.

Respectfully submitted,

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High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids

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Abstract

The concepts of high-throughput (HT) screening and combinatorial synthesis have been integrated into the pharmaceutical discovery process, but are not yet commonplace in the pharmaceutical development arena. Emerging strategies to speed pharmaceutical development and capture solid form diversity of pharmaceutical substances have resulted in the emergence of HT crystallization technologies. The primary type of diversity often refers to polymorphs, which are different crystal forms of the same chemical composition. However, diverse salt forms, co-crystals, hydrates and solvates are also amenable to study in HT crystallization systems. The impact of form diversity encompasses issues of stability and bioavailability, as well as development considerations such as process definition, formulation design, patent protection and regulatory control. This review highlights the opportunities and challenges of HT crystallization technologies as they apply to pharmaceutical research and development.
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Keywords: High-throughput; Crystallization; Polymorph; Solvate; Salt; Co-crystal

Contents

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1. Introduction

Active pharmaceutical ingredients (APIs) are frequently delivered to the patient in the solid-state as part of an approved dosage form (e.g., tablets, capsules, etc.). Solids provide a convenient, compact and generally stable format to store an API or a drug product. Understanding and controlling the solid-state chemistry of APIs, both as pure drug substances and in formulated products, is therefore an important aspect of the drug development process. APIs can exist in a variety of distinct solid forms, including polymorphs, solvates, hydrates, salts, co-crystals and amorphous solids. Each form displays unique physicochemical properties that can profoundly influence the bioavailability, manufacturability purification, stability and other performance characteristics of the drug [1]. Hence, it is critical to understand the relationship between the particular solid form of a compound and its functional properties. Discovery and characterization of the diversity of solid forms of a drug substance provide options from which to select a form that exhibits the appropriate balance of critical properties for development into the drug product. Importantly, the desired properties may vary with each mode of delivery (i.e., oral, pulmonary, parenteral, transdermal, etc.), such that the solid form may differ for each optimized dosage form. Given these options, the choice and design of pharmaceutical solid forms can be critically important to successful drug development.

Solid form discovery and design depends on the nature of the molecule of interest and type of physical property challenges faced in its development. The preferred solid form is generally the thermodynamically most stable crystalline form of the compound [1,2]. However, the stable crystal form of the parent compound may exhibit inadequate solubility or dissolution rate resulting in poor oral absorption, particularly for water-insoluble compounds. In this case, alternative solid forms may be investigated. For ionizable compounds, preparation of salt forms using pharmaceutically acceptable acids and bases is a common strategy to improve bioavailability [1,3,4].

Like the parent compound, pharmaceutical salts may exist in several polymorphic, solvated and/or hydrated forms.

Most APIs and their salts are purified and isolated by crystallization from an appropriate solvent during the final step in the synthetic process. A large number of factors can influence crystal nucleation and growth during this process, including the composition of the crystallization medium and the process(es) used to generate supersaturation and promote crystallization [1,5–13]. The most notable variables of composition and processing are summarized in Table 1. Solid form screening is used to understand the effects that these variables have on the polymorphic outcome of a crystallization experiment, so that a robust process can be identified to produce the desired crystal form. Traditionally, the study of solid form diversity of active compounds has relied on the use of a variety of common process methods for generation of new forms, coupled with modern characterization methods for analysis of the solids produced [2,14]. Most often, however, a combination of solvent recrystallization (cooling or evaporative, as well as slurry conversion) and thermal analysis (e.g., hot stage microscopy, differential scanning calorimetry) are employed for initial form screening. Such methods are inherently slow and only allow exploration of a small fraction of the composition and process space that can contribute to form diversity. Before suggesting a form for development, scientists may have carried out only a few dozen crystallization experiments and possibly prepared a handful of different salts of a compound. The main reasons for the limited number of experiments are the constraints on availability of compound and scientists' analytical capacity in a given time frame, and they are therefore often forced to make form selection decisions on incomplete data. Accordingly, it is not surprising that unexpected and undesired outcomes can, and do, occur later on in development.

Despite more than a century of research [15], the fundamental mechanisms and molecular properties that drive crystal form diversity, specifically the nucleation of polymorphic forms, are not well under-

Table 1
Crystallization composition and processing variables [1,2,8]

Composition type		Process variables[a]				
Polymorph/ solvates	Salts/ co-crystals	Thermal	Anti-solvent	Evaporation	Slurry conversion	Other variables
■ Solvent/ solvent combinations	■ Counter-ion type	■ Heating rate	■ Anti-solvent type	■ Rate of evaporation	■ Solvent type	■ Mixing rate
■ Degree of supersaturation	■ Acid/base ratio	■ Cooling rate	■ Rate of anti-solvent addition	■ Evaporation time	■ Incubation temperature	■ Impeller design
■ Additive type	■ Solvent/ solvent combinations	■ Maximum temperature	■ Temperature of anti-solvent addition	■ Carrier gas	■ Incubation time	■ Crystallization vessel design (including capillaries, etc.)
■ Additive concentration	■ Degree of super-saturation ■ Additive type and concentration ■ pH ■ Ionic strength	■ Incubation temperature(s) ■ Incubation time	■ Time of anti-solvent addition	■ Surface-volume ratio	■ Thermal cycling and gradients	

[a] Applicable to all types of screens.

stood [13,16]. As a result, predictive methods of assessing polymorphic behavior of pharmaceutical compounds by ab initio calculations remain a formidable challenge. Even in cases where the existence of a crystalline form is predicted, the stability relative to other crystalline packing arrangements has been difficult to estimate with accuracy [17]. Moreover, the prediction of packing structures for multicomponent (e.g., solvates, hydrates, co-crystals) or ionic systems is not yet possible [17]. Due to these limitations, solid form discovery remains an experimental exercise, where manual screening methods are employed to explore form diversity of a compound.

Control over solid form throughout the drug development process is of paramount importance. Reliable preparation and preservation of the desired form of the drug substance must be demonstrated, and has become increasingly scrutinized by regulatory agencies as more sensitive and quantitative solid-state analytical methods have become available [18]. Many strategies to influence and control the crystallization process to produce the solid form of interest have been reported. Some examples include stereochemical control using tailor-made auxiliaries [19–21], targeted solvent recrystallization [22–24], and templating using a variety of surfaces (e.g., organic single crystal substrates [25], surfaces of metastable crystal faces [25,26], inorganic crystal

surfaces [27] and polymeric materials [28]). Recent studies have also begun to uncover the role of reaction byproducts and other impurities in determining polymorphic outcome and crystal properties [29–32], and in fact, it has been shown that in some cases such species can stabilize metastable crystal forms [33,34]. In addition, new processing methods continue to be developed to improve discovery and characterization of new forms, including precipitation by supercritical fluid [35,36], laser induced nucleation [37–39] and capillary crystallization [40–42]. However, there remains a lack of fundamental understanding of the nucleation process and the specific factors that contribute to crystallization of diverse forms of a compound [13,21,23]. In order to fully control the crystallization process, the link between the physical or chemical processes that influence nucleation and crystal growth needs to be better established. It is in this area that new experimental methodologies have the potential to enable development of this knowledge base.

There is reason to believe that the already complicated landscape of pharmaceutical solid forms will become even more complex in the future. It is now increasingly appreciated that hydrogen bonded co-crystal structures between active agents and molecules other than water or solvent can be prepared. For example, co-crystals of aspirin, rac-ibuprofen and

rac-flurbiprofen have been prepared by disrupting the carboxylic acid dimers using 4,4′ -bipyridine [43]. These structures are formally molecular compounds (or co-crystals) but do not involve formation of covalent bonds or charge transfer from or to the active substance. Recent demonstrations of these principles with drug compounds have been published [43–45].

Exploration of a given compound's polymorphs, hydrates, solvates, salts, co-crystals and combinations of all of these appears intractable by conventional experimental methods, and as the number of potential methods for exploring and controlling crystal form diversity continue to expand, existing strategies will become increasingly inadequate. In an effort to understand form diversity in a more comprehensive manner, high-throughput (HT) crystallization systems have recently been developed. This methodology uses a combinatorial approach to solid form generation, where large arrays of conditions and compositions are processed in parallel. Experiments are performed at small scale to reduce the material demand and to afford the largest number of conditions possible. The large number of crystallization trials performed in these experiments reflects the reality that nucleation rate has an extremely non-linear dependence on the experimental conditions, and as such, the probability of a chance occurrence of a particular form is increased by a HT approach. Supersaturation (solubility) and induction time of the various possible solid forms are independently controlled by these conditions, resulting in highly non-linear time dependence of crystallization. In addition, the combinatorial approach permits exploration of a chemical continuum, where use of many solvent mixtures may allow one to assess what underlying physical or chemical processes are required to produce a particular solid form. Once a variety of conditions that can be used to produce a given crystal form on the microscale are identified in the HT screen, scale-up studies are typically conducted to optimize the process for laboratory scale production.

In this review, the development and application of novel HT crystallization technologies for exploration of solid form diversity are discussed. The operational features of a fully integrated, automated HT crystallization system are presented, highlighting the design requirements for hardware and software components, as well as general specifications for consumables.

Case studies are used to illustrate the benefits and capabilities of the approach, including salt selection in early lead optimization (ELO) and pre-clinical development, polymorph and solvate screening in highly polymorphic systems, comprehensive discovery of crystal forms to reduce the risk of late displays of polymorphism, comparison of experimental and predictive methods of solid form discovery, and engineering of co-crystals. The need for post-screening characterization of crystal forms to enable ranking and selection of the most suitable form for development is briefly reviewed. Finally, the implications of HT crystallization technologies on the future of solid form screening processes, intellectual property protection and regulatory compliance are discussed.

2. Development of high-throughput crystallization technologies

HT crystallization systems have been developed to more rapidly and comprehensively explore the multi-parameter space that contributes to solid form diversity [40,46–51]. In its simplest description, HT crystallization can be broken down into three key experimental steps: *design* of experiment (DOE), *execution* of experimental protocols and *analysis* of data. Systems designed to carry out these experiments generally consist of both hardware and software components that drive and track experimentation, and permit data storage, retrieval and analysis. Such systems should be designed to be flexible and scalable to ensure that a variety of experimental procedures can be carried out either serially or concurrently. Thus, the system can be employed at various stages of drug development, where differences exist in the quality and quantity of compound available. While it is highly desirable to have the ability to mine and model experimental data, and to use the subsequent knowledge to guide further experiments, not all HT crystallization systems are equipped with these features. In Section 3, the hardware and software considerations for design and development of a fully integrated, informatics-driven HT crystallization system are described.

While the concepts of HT screening are widely applied in the pharmaceutical industry, most notably in the drug discovery arena [52], the application of

HT approaches to drug development, in particular solid form screening, are just beginning to be realized. These latter approaches, however, are more akin to HT experimentation than HT screening. Hence, several important distinctions, which reflect on the design of HT experimental systems, need to be made. First, the goal of HT screening is to get a small number of successful outcomes, which are then passed on to the next stage of development. Little effort is typically made to learn why certain outcomes were positive and why others were negative. In contrast, HT experimentation, such as HT crystallization, is carried out with the goal of having each point in the experiment produce multiple types of data that can be interpreted, and the interpretation used to guide the experimental process to a successful conclusion. Second, unlike traditional HT screening assays where experiments are generally conducted under constant experimental conditions, HT crystallization experiments for solid form discovery are best conducted using a variety of process methods, each having varying experimental conditions (e.g., temperature variations as a function of time) over the course of the experiment. These additional process variables permit maximal diversity in the experimental space, increasing the likelihood that comprehensive coverage will be achieved. Finally, there is a distinction to be made in terms of relative "hit rates". In both HT screening and HT crystallization, a "hit" can be

thought of as a set of conditions that gives rise to a desired result. In HT screening, the desired result is typically an activity, or potency, that exceeds a predefined threshold. In HT crystallization, a hit is defined as the formation of a solid. The typical observed hit rate of HT screening is on the order of 0.1% of the total number of samples analyzed. In contrast, HT crystallization experiments can yield hit rates ranging from tens of percents to nearly 100%, depending on the type of experiment and the process mode(s) used. For example, while only a handful of compounds from a selection of thousands may exhibit the required potency, 10–50% of crystallization trials may yield solids. In fact, the range of wells that yield solids is very wide, depending on process mode and experimental time scale, as will be discussed in subsequent sections. The impact of these differences is manifested in the design and operational requirements of HT experimentation systems.

A fully integrated HT crystallization system consists of a number of components, including experimental design and execution software, robotic dispensing and handling hardware, automated high-speed micro-analytical tools, end-to-end sample tracking and integrated cheminformatics analysis software for data visualization, modeling and mining. A schematic overview detailing the workflow of such a system is depicted in Scheme 1 [53]. These features are supported by a comprehensive informatics foun-
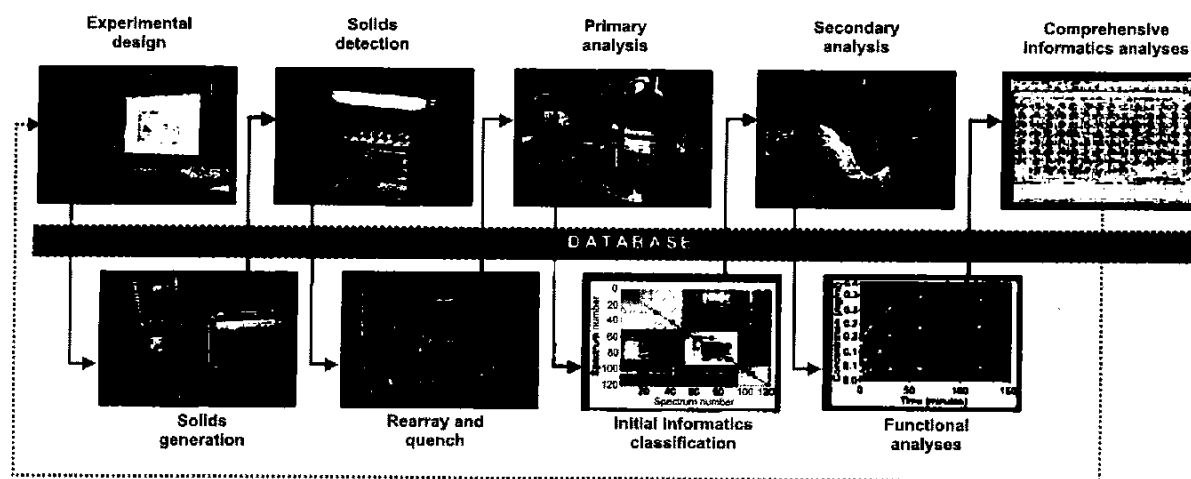
Scheme 1. A schematic illustration of the workflow of a fully integrated HT crystallization system [53].

dation that is used to handle the large quantities of data generated. Specifically, informatics tools are used to design statistically relevant and diverse experiments, drive the automation hardware to perform the specified operations, and provide an analytical function to analyze, compare and sort the results of experiments. An important feature of these systems is the ability to mine and model experimental data and use the knowledge generated to guide further experiments. These functions are supported by use of a relational database that provides a mechanism of communication between system components.

When designing a HT crystallization experiment, or set of experiments, a large variety of parameters of composition and process are involved. Experimental designs must be aimed at covering a large multifactorial parameter space, with the goal of determining which experimental factors affect the desired outcome. In practice, it is desirable to place constraints on the experimental space, making common statistical design methods such as full or partial factorial designs inappropriate or impractical. For example, hardware limitations, including minimum and maximum dispense volumes or masses and accessible temperature ranges, as well as constraints related to chemical compatibility (i.e., reactivity of components, miscibility, etc.) or toxicity limits of components (if appropriate), need to be considered. Thus, alternative DOE methods that can accommodate such constraints are required. D-optimal design [54,55] is an example of a DOE algorithm that can take a set of constraints, such as the ones described above, in combination with a target analytical model and determine the optimal set of experimental points to test. Another commonly used DOE algorithm is diversity generation, with which the experimentalist selects a set of pertinent chemical properties and uses the algorithm to evenly spread experimental points over the chosen property space. In addition, some systems utilize a solubility calculator tool to estimate the solubility of the API in the given solvent/additive mixture. The calculated information is then used to select the appropriate concentration of API in each mixture so that it is supersaturated with respect to the reference phase at the harvest temperature. Here, the driving force for crystallization can also be varied by tailoring the composition of each sample based on the API solubility in that mixture. With such DOE tools, experiments may be designed to effective-

ly and simultaneously explore the diverse composition and process space described in Table 1.

Ideally, DOE algorithms should also incorporate prior knowledge or experimental results, which have been stored in a database as a set of rules or models, to limit an experimental space to have certain predicted characteristics. For example, over the course of time, a regression model may be developed between a set of known or calculated chemical properties and a parameter of experimental interest. The model could be used during the design of a new experiment in order to test only those chemicals that are predicted to give a desirable result. Since a large number of factors need to be considered during experimental design, the DOE interface available to the scientist must not only be flexible and easy to use, but must also offer tools that aid design efficiency and effectiveness and permit input of scientific knowledge generated over time.

At the end of the experimental design process, the resulting set of experimental conditions is translated into a series of commands for the HT systems, and stored in a relational database for later retrieval by the software that controls the automation. When an experiment is activated, the overall operation of the automation systems is managed by the HT informatics system, which is responsible for physical operation of the HT platforms as well as data tracking and storage.

Execution of experimental commands is carried out by automated laboratory equipment that comprises the HT crystallization system. Specialized automated systems perform several of the functions in a sequence of events that make up the experiment. Each station is controlled through an interface to the informatics system that ensures the samples are processed at the correct stations, in the correct order, with the selected experimental parameters being followed. Parameters of operation are recorded, including the time at which an action is taken. After execution of the experimental steps, the software interface retrieves any pertinent information generated by the automated platform, such as assay results or operational parameters, stores these data in the relational database, and updates the status of the experiment to reflect the completion of operations.

In general, the hardware required for a HT crystallization system is comprised of four major functional elements: sample preparation, solids generation, solids detection and sample analysis. Sample preparation

involves adding the compound of interest (API) to the diverse set of conditions used to conduct crystallization studies. Typically, the API is dispensed as a solution in a suitable solvent, followed by solvent removal to yield the solid API. Solvent removal can be achieved by passive evaporation or by controlled active evaporation (e.g., use of a vortex dryer). Alternatively, the API can be delivered in the solid state with suitable powder handling systems. Depending on the amount of saturation desired, the crystallization vessel used, and the API's solubility in solvents or solvent mixtures of interest, API masses ranging from a few hundreds of micrograms to several milligrams will be present in each vessel. Once the API has been delivered to the crystallization vessels (tubes, vials or microwell plates), combinations of solvents and/or additives are added to each vessel. By taking advantage of the power of combinatorial approaches, large numbers of unique combinations can be dispensed from manageable sets of starting materials.

Compatibility of equipment components (syringes, dispense tips, tubing, etc.) and consumables (plates, tubes, etc.) with solvents and other compounds is a key hurdle faced in the development of combinatorial crystallization for small molecules. Unlike protein crystallization systems [56,57], which are commonly based on the sitting-drop method in aqueous media, small molecule crystallization employs a range of crystallization additives and processes. The additives include organic solvents with varying properties (e.g., alcohols, acetone, hexane, ethyl acetate, etc.), water, acids, bases and co-crystal formers, as well as other compounds (e.g., small molecule templating agents, surfactants, pharmaceutical excipients, etc.). This wide range of materials needs to be handled by appropriate liquid handling techniques to enable the combinatorial assembly previously mentioned. Ideally, liquid transfers are achieved using multichannel pipettors with individually controllable channels. Depending on the crystallization vessel design, the volumes of reagents dispensed will be as low as a few microliters to as high as several hundred microliters.

Potential for cross-contamination and tendency toward unwanted solvent evaporation from crystallization wells are challenges that need to be addressed in a HT crystallization system. A large number of the solvents used to crystallize small molecules have high vapor pressure under ordinary laboratory conditions. Sealing of the crystallization vessels is key to being able to control composition during crystallization from these solvents. Due to solvent fugacity, vessels need to be protected from ingress of the components of neighboring wells. These problems have been solved by different means, such as sealing of individual tubes with a Teflon-backed crimp seal [40] or O-rings/gasket seals and clamped covers [47,51].

HT crystallization must enable several process modes that are compatible with the compound (e.g., chemical stability, thermal stability, etc.). In some cases, multiple modes of operation may be combined. The most common modes of solids generation will be discussed below, including thermal cooling crystallization, anti-solvent and evaporative crystallization. Less common process modes include melt crystallization, flash or quench cooling and template-directed crystallization. It is important to note that generation of maximal diversity in solid form requires multiple modes of operation [6,18,58].

In thermally induced cooling crystallization, samples created in the sample preparation process described above are subjected to temperature ramps. Prior to beginning the temperature ramp, samples are exposed to an elevated temperature for a short period of time in order to dissolve the API in the crystallization medium. Although dissolution can be achieved most simply by diffusion and convection from the heating process, addition of external energy can speed up the process (e.g., sonication). Samples may be optically inspected (see Fig. 1) and vessels that contain undissolved solids can be flagged in the database for further analysis. For instance, undissolved samples may be treated as slurry conversion experiments and monitored over time for crystal form changes. The thermal cycle is then initiated, using controlled cooling to induce supersaturation. In this mode of crystallization, samples continually experience an under cooling and, based on the level of supersaturation in the vessel, may recrystallize at a given temperature after a period of time. Thermal crystallization tends to generate a cumulative number of samples that are produced over time in a fashion approximating a square root function, as illustrated in Fig. 2. This means that initially there is a small bolus of "hits", after which the rate of crystallization tails off over a period of time, typically in days to weeks. This results in a manageable hit rate

Fig. 1. Photo of optical inspection station. (Inset shows close up of crystallization vessel that contains crystals.) (Courtesy of Trans-Form Pharmaceuticals, 2002.)

for analysis, on the order of approximately 10% in aggregate. This mode of solids generation has the lowest throughput rate, typically, because experiments span days to weeks, with system residence times of months being possible.

In contrast, anti-solvent addition, also known as "crash-out" (or "drown out") crystallization, relies on the fact that an API is soluble to varying degrees in the crystallization medium, but is largely insoluble in a particular solvent or solvents (e.g., the anti-solvent). As a result, this mode of crystallization can operate at high-throughput rates, with samples being turned around hourly. When crystallization vessels containing API in reagent mixtures are exposed to aliquots of anti-solvent, nearly all vessels will contain API that has precipitated out of solution. This creates a challenge to the analytical process, as the near 100% hit rate leads to a large bolus of samples. There are, however, advantages to this mode of solids generation, such as the ability to produce microfine crystallites and amorphous solids, should they be desired.

Lastly, evaporative crystallization can be carried out on the combinatorial array of samples. This mode of operation relies on gradually increasing the concentration of API in the vessel to achieve supersaturation and to increase the degree of supersaturation (by preferential evaporation) in order to induce crystallization. Concentration of samples can be achieved either passively or actively by controlled flow of inert gas while maintaining temperature. With evaporative

methods, differential rates of solvent loss from mixtures result in unknown composition of the crystallization medium at the time of crystal nucleation. In addition, the degree of supersaturation changes over the course of the experiment, often resulting in the appearance of multiple crystal forms. The evaporative mode of solids generation typically produces throughput and hit rates intermediate between the thermal and anti-solvent processes.

As suggested above, in appropriately configured HT crystallization systems, several process modes may be used in series or in parallel [40]. Frequently, the preparation of replicate plates (in some systems "daughter" plates [47,51]) is necessary for parallel processing by different process modes. Systems may be additionally equipped with the ability to serially process sample arrays using different process modes [59]. This feature is particularly attractive for cases where only small quantities of sample are available, increasing the drive to generate useful information from every sample. Here, samples may be processed by optimal modes first (e.g., thermal crystallization), then a secondary process step can be applied to maximize the hit rate. Another example where this feature is useful is in the case of salt selection, especially in early drug discovery. Upon the addition of salt forming acids or bases, the solubility of the compound is modulated by in-situ salt formation, often resulting in reduced or non-existent driving forces for crystallization (e.g., subsaturation) of the salt species, particularly in polar
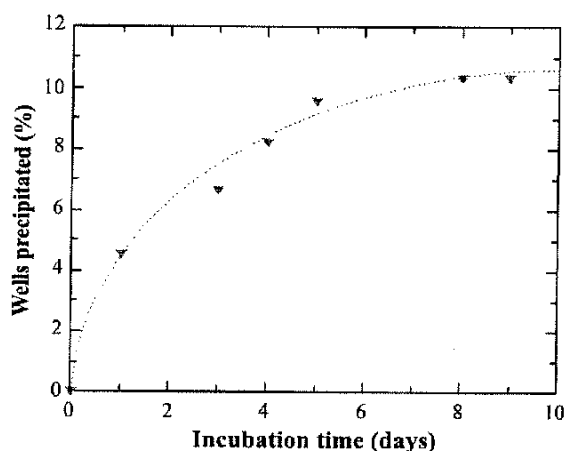

Fig. 2. Typical rate of appearance of solids during a thermally driven HT crystallization experiment [65].

solvents. It should be noted that rapid onset of super-saturation can be experienced in any of the process modes discussed and can result in oiling out or precip-itation of amorphous solids, rather than generation of crystalline solids. Thus, it is important to monitor and control the crystallization conditions throughout the experiment.

In general, the percentage of wells that yield solids varies, depending on process mode and experimental time scale. For example, evaporative modes usually result in a solid in virtually every vessel, while slow undercooling results in far fewer (on the order of low percents). The differences in hit rates between these process methods arise in part from the differences in the supersaturation attained. For evaporative crystalli-zation, supersaturation is achieved in all cases as the concentration of the active compound is continuously increased as solvent is evaporated. In contrast, the composition of wells processed by thermal crystalli-zation is fixed. In some cases, because there is limited data on the precise state of supersaturation for each of the large variety of experimental compositions and potential crystal forms, some wells may remain subsaturated during the process. For these wells, additional process steps, such as partial evaporation or anti-solvent addition, may be employed to generate supersaturation to yield a solid. In contrast, as men-tioned previously, a fraction of the wells may not go fully into solution at elevated temperatures. In this case, the temperature of the system may be raised to achieve full dissolution, additional solvent may be added to solubilize residual solids or the samples may simply be monitored for slurry conversion over time. To overcome these challenges, we have devel-oped a solubility calculator tool using group contribu-tion theory to estimate the solubility of the reference solid phase at specified temperatures in each solvent composition. These data are then used at the DOE step to define the viable concentrations of the active com-pound for crystallization (i.e., minimum concentration required to achieve saturation and maximum solubility limit or concentration) in each solvent mixture. Addi-tionally, the timescale of the experiment has a signif-icant impact on the observed hit rate. Hit rates will approach 100% for viable crystallization conditions in the limit of infinite time, but in practice most experi-ments are conducted over days to weeks, so observed hit rates reflect this temporal influence. In fact, similar

behavior is observed in manual experimentation. Note that only some HT crystallization systems are config-ured to permit selective sampling of "hits", providing the ability to further incubate un-crystallized samples to monitor for slow growing crystal forms.

Solids detection can be achieved by examining each sample using machine vision systems. Samples may be monitored over time to detect precipitation in vessels that were previously devoid of solids. This simple, yet robust process can rapidly and non-destructively de-termine state changes in the crystallization vessels and signal when a particular vessel or set of vessels is ready for solid-state analysis. Depending on the sample array configuration, the signaling of "hits" results in har-vesting of samples by one of two approaches. In the "cherry-picking" approach, only those samples that have been flagged as containing solids are selected for further processing [40]. In contrast, using a sacrificial approach the entire plate must be moved forward after a predetermined fraction of the samples in that array have produced precipitates [47,51]. The latter, of course, can be carried out without an online detection system. Here, samples can be processed in batches, without regard to whether there are actually solids present in a vessel. This simple process approach is effective, but has significant limitations, the primary of which being that samples are destroyed after a fixed amount of time regardless of their state. Hence, it is advantageous to employ an online detection and har-vest system so that samples can be differentially and asynchronously processed, with only those vessels containing solids undergoing analysis [40,60].

Sample analysis is the final action in execution of the HT crystallization process. Depending on the mode of operation and the choice of analytical measurements employed, this process may involve several steps. Most HT crystallization systems use Raman spectroscopy and/or powder X-ray diffraction (PXRD) for primary analysis of harvested solid-state samples. Both techni-ques have advantages and disadvantages in terms of their ability to discriminate between forms of a solid (i.e., polymorphs, salt forms, solvates, hydrates) [1,14,61]. The rate of generation of samples for anal-ysis likely dictates which technique is used for the primary approach. Generally speaking, Raman spec-troscopy can be employed in a more rapid fashion than PXRD, since acquisition times for Raman are consid-erably less dependent on sample size, as is depicted in
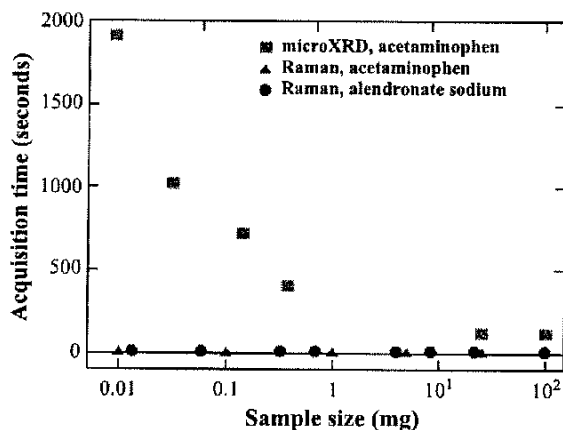
Fig. 3. Comparison of acquisition times of Raman and X-ray powder diffraction data as a function of mass of API [65]. (Data collected on D/Max Rapid, Contact Rigaku/MSC, 9009 New Trails Drive, The Woodlands, TX, USA 77381-5209).

Fig. 3. In addition, plate-based PXRD methods are susceptible to problems with preferred orientation effects, which may prevent accurate classification of samples. As a result, Raman spectroscopy methods are often used as a primary means of characterization in HT crystallization systems. Although one disadvantage of the Raman technique is interference due to fluorescent samples, the wavelength of the excitation laser can be changed to the near-IR to reduce fluorescence of problematic samples. Recent advances in PXRD instrumentation, brought on by the increasing demands of HT crystallization, make it possible to achieve similar analysis timescales with PXRD and Raman, on the order of less than one minute per sample depending on the capabilities of particular instruments used. Clearly, the best option is to employ both methods for initial sample evaluation, which can be realized with the appropriate informatics structure, as described in Section 3.

Once the primary solid-state characterization data are collected and stored, samples are generally classified into groups (or bins) that display similar characteristics (e.g., Raman spectra or powder X-ray diffraction patterns) using informatics tools. A variety of methods can be used to accomplish the binning. For instance, Raman spectra may be compared (based on relevant features or over the entire spectral range) and clustered using calculated similarity measures, such as Tanimoto coefficients. In one method [40,60,61], each Raman

spectrum, which represents the contents of an individual well at a given time, is filtered to remove background and to accentuate Raman peaks and shoulders. Peaks are then located and assigned a wavenumber using standard derivative methods and the amplitude of each peak is calculated. These data are used to calculate a similarity (or distance) measure related to the Tanimoto coefficient, from which the Raman spectra are binned into groups of similar samples using a classification algorithm such as hierarchical clustering. This method often uses peak positions, rather than amplitudes to discriminate between different patterns in order to reduce the significance of potential preferred orientation effects, which can result in modulation of relative peak intensity for certain crystallographic planes. The window over which two peaks are considered to be at the same position (e.g., 1 cm^{-1} wavenumber), as well as a minimum height for a filtered peak to be considered for clustering, can be selected by the user, allowing regions of interest (e.g., spectral ranges) to be explored in greater detail. With appropriate settings, a Raman spectrum that has only one peak or feature in a slightly different location than observed in other patterns can be differentiated and binned as unique, indicating a different or new crystal form. During clustering, each spectrum is assigned an arbitrary number, i.e., a sorted spectrum number, for ease of tracking, and the resultant clusters are graphed as shown in Fig. 4, where the red-colored regions repre-
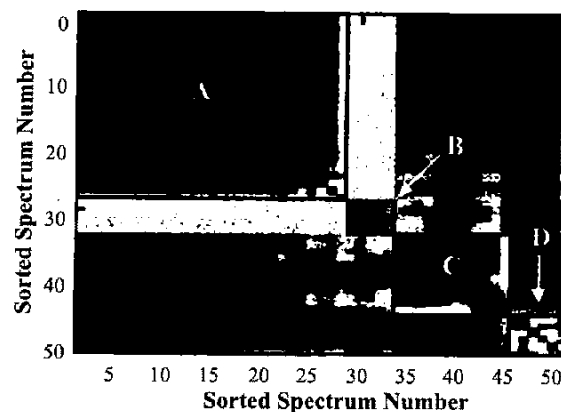

Fig. 4. Raman cluster diagram showing n-by-n matrix of sorted spectrum numbers for all samples resulting from the HT polymorph screen of Ritonavir. Clusters are indicated by warm-colored (red) regions, which have been outlined to guide the eye, and indicate different solid forms [65].

sent bins of similar samples. Alternatively, the results from several analytical methods such as Raman and PXRD can be used to simultaneously classify samples.

Regardless of the choice of primary analytical method, and in keeping with traditional methodologies for solid form screening, it is necessary to further characterize the solids generated in HT crystallization systems to accurately determine their solid form and properties. Most HT systems integrate multiple analytical methods as part of the screening process. These so-called secondary analytical methods often include thermal property measurement (e.g., melting point) and optical microscopy (for crystallinity, habit, etc.). Depending on how the samples are processed and the degree of computerized support, these techniques may be applied to all samples, or a subset of selected samples. For systems that analyze all samples by secondary techniques, several HT plate-based methods for optical microscopy and melting point determination have been developed [47,51]. It is important to note that, in this case, all samples are destroyed during characterization of the melting point. When replicates are retained, the functional properties such as dissolution rate and hygroscopicity can be analyzed using either manual or HT methods. (For more information on functional analysis, see Section 4 on post-screening analyses and form selection.)

With the aid of informatics tools, the data sets obtained can be used to generate information about the experimental space. Software interfaces that allow access to the data permit classification and regression analysis to be performed. The results are displayed in high-dimensional visualization tools that can be used to guide further experiments toward optimizing processes to make each form. For instance, sample composition and processing information can be linked to the resulting crystal form and morphology. Correlation of trends between experimental factors and the products can lead to hypotheses that can be used to direct the design of follow-up experiments. An example of this was reported by Peterson et al. [40], where the knowledge gained from iterative experiments was used to drive new experimental designs, which ultimately yielded the desired outcome, i.e., the isolation and characterization of the highly unstable form III of acetaminophen (paracetamol).

While these new methodologies provide unprecedented capabilities for solids form discovery, it is clear

that there remains a need for some level of manual processing, particularly in the case of detailed form characterization such as single crystal structure determination, scale-up of the desired form and understanding the effects of downstream processing on potential form conversion. HT methods provide the landscape of possible forms and their properties and should be used in conjunction with traditional methods to enable rapid, efficient selection of the optimal form for development.

3. Applications of high-throughput crystallization screening in pharmaceutical research and development: case studies

HT technologies offer unprecedented capabilities for form discovery and characterization. Potential applications range across the entire pharmaceutical value chain, including screening of active molecules in discovery during ELO, form selection for preclinical candidates, final form optimization for early clinical candidates, process chemistry development of crystallization processes for bulk drug and intermediates, as well as identification of new or enabling solid forms for product life cycle management. While numerous impact points have been identified, only limited information on the use and performance of HT form screening systems is available in the literature, indicating that the benefits of these new methodologies have just begun to be realized. In the following sections, case studies on the application of HT crystallization systems are reviewed. Special attention is given to the implications of new form discoveries.

3.1. High-throughput salt selection

Preparation of salt forms of an active compound is commonly used to modulate physicochemical properties. In most cases, the goal is to increase solubility (or dissolution rate) to improve bioavailability or to enhance the manufacturability of poorly soluble ionizable compounds [1,3,4]. Salts may also be employed to increase chemical stability [3] or to reduce the solubility of a given compound for certain applications (e.g., sustained release dosage forms) [62]. Thus, it is important to consider the route of administration and

dosage form requirements when selecting a salt form for development. Since the choice of counter-ion affects the properties of salt forms [3,4], salt selection studies involve the preparation of a number of different salts using a variety of pharmaceutically acceptable acids or bases with differing properties (e.g., acidity/ basicity, molecular size, shape, flexibility, etc.). The relevant physicochemical properties of each salt are characterized, including degree of crystallinity, hygroscopicity, aqueous solubility, crystal habit, and physical and chemical stability. Based on these properties of the salt forms, their suitability for development can be evaluated. Several strategies for streamlining and optimizing salt selection procedures have been reported, including in-situ techniques for ranking the solubility of salts [63], tiered approaches in which the least time-consuming studies are carried out first and used to remove from consideration salts that are not viable [64]. One issue not readily considered by existing strategies is the polymorphism and solvate forming behavior of the different salt forms of a compound, which could be used as an additional criterion when more than one salt may be viable, but the degree of polymorphism and solvate formation of each may become a criterion for form selection.

HT crystallization technologies have been used to more rapidly and comprehensively identify the range of salt forms that may be prepared for a given compound or series of compounds, and characterize their crystal form diversity (polymorphs, solvates, hydrates). However, only a few studies have been published or presented. Several HT salt selection studies on well-characterized pharmaceutical compounds have been carried out to demonstrate the power of these technologies in solid form discovery. For example, in a small HT study (i.e., 96 wells) on the antibacterial sulfathiazole, salt formation was explored using varying stoichiometric ratios of pharmaceutically acceptable organic and mineral bases in an array of solvent conditions [65]. The screen resulted in the rapid identification and characterization of 10 salt forms and showed that the salts exhibited a range of melting points depending on the counter-ion type and stoichiometric ratio. Similar HT salt selection experiments on caffeine and naproxen resulted in the identification of numerous salts of each compound [47,50,51].

In the discovery phase, HT crystallization has been used to identify soluble salt forms of compounds during ELO to facilitate early animal dosing, thereby providing the ability to uncover underlying chemical and/or biological responses elicited by candidate molecules, including toxicity or efflux [46,59]. Such information permits rapid identification of problematic compounds or scaffolds, allowing resources to be directed to projects with greater opportunity for success. HT crystallization can facilitate selection of leads that are more likely to survive preclinical development. HT crystallization has been used successfully to identify multiple new salt forms and the polymorphs and solvates of each compound belonging to two discovery programs using less than 200 mg of compound per screen [59]. Approximately 150–200 experiments were performed on each compound using a library of pharmaceutically acceptable acids or bases with an array of solvent compositions and process conditions. Each screen resulted in discovery of multiple new salt forms, and in some cases polymorphs and solvates. Interestingly, similar salt types were identified for each compound in a given series, as illustrated in Fig. 5, where the frequency of occurrence is plotted as a function of counter-ion for each discovery series. Clear trends in the degree of solid form diversity of salt forms, including polymorphism and solvation behavior, were also evident within each compound series. These data indicate the potential for identifying salts suitable for most compounds tested in a particular scaffold or series, based on analysis of only a portion of the series, i.e., a platform-based approach to salt selection, provided the chemistry surrounding the ionizable functionality is not significantly altered during further structure–activity relationship (SAR) development. Furthermore, solubility measurements of each salt form in physiologically relevant fluids allowed ranking of salt forms in a given series, and comparison of salts between series was also possible. The average turn-around time per screen was approximately 2 weeks, such that feedback on the physicochemical properties of each compound was provided to the medicinal chemists on a similar time scale as potency, selectivity and metabolism screens.

Salt selection is normally part of the standard preformulation studies carried out during preclinical development, where rapid identification of the possible salts of a compound and their properties can facilitate product development. To further facilitate
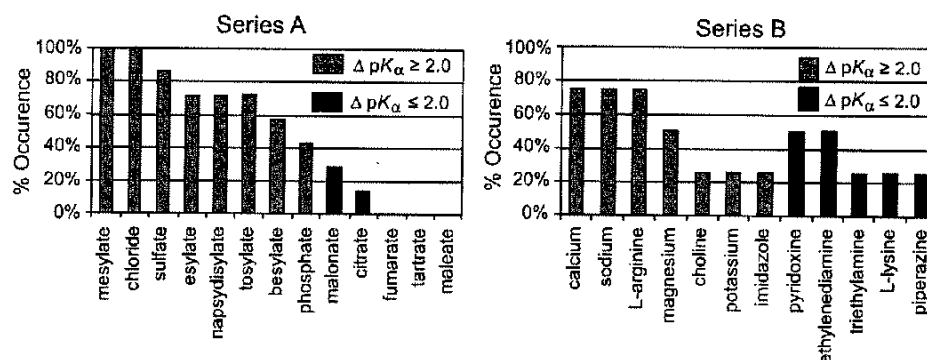
Fig. 5. Frequency of occurrence (%) plotted as a function of the counter-ion of the salt for compounds from discovery series A and B [59].

such studies, a microplate technique capable of investigating an array of conditions has been developed to determine which counter-ion and solvent conditions can be used to prepare crystalline salts of the compound [66]. Each plate is prepared by first depositing approximately 0.5 mg of compound into each well using an appropriate amount of stock solution. The counter-ion type is systematically varied along the rows of the plate and different crystallization solvents are deposited down the columns of the plate. Crystallization is monitored by optical microscopy over the course of the evaporative crystallization, which can be accelerated by flowing a stream of dry nitrogen over the plate. Once salt forms are identified, they are scaled up for more detailed characterization.

The microplate approach was demonstrated by Bastin et al. [66] through several examples, however little detail of the specific screening protocol and results was provided. All three of the reported examples are on compounds that are weak bases with pK_a between 4.1 and 5.3. Only a small number of stable, crystalline salts could be prepared for the two very weak bases (i.e., $pK_a < 4.25$), as opposed to the larger variety found for the stronger base. In each case, the salt forms were scaled-up for more detailed analysis and comparison to the respective free base compound to determine the optimal form for development. This approach provides a useful mechanism for preliminary, small-scale salt formation studies. Both the crystallization media and process modes accessible by the technique are somewhat limited, resulting in a narrow exploration of experimental conditions for salt

formation. For example, only solvents compatible with plate materials can be used, thereby reducing the probability that a crystalline phase can be identified. In addition, current protocols only provide for evaporative crystallization, likely due to difficulties with sealing of the plates. In this case, the composition of the crystallization medium is not well controlled. The utility of HT crystallization in ELO, although demonstrated by initial reports of feasibility, is less well documented than the use of HT on later stage compounds.

3.2. Solid form discovery in highly polymorphic systems

The statement by the late Walter McCrone in 1965 that "the number of forms of a given molecule is proportional to the time, money and experiments spent on that compound" [67] has gained credence in recent years, as illustrated by the significant increase in reported crystal form diversity of pharmaceutical solids. Depending on when alternative solid forms of a compound are identified, the appearance of a novel form may or may not be a welcomed discovery. Occurrence of a new form in research or early development is potentially enabling. At later stages, the appearance of new forms, particularly stable ones that are not bioequivalent or deemed unprocessable, can have catastrophic consequences for product performance as well as regulatory compliance (e.g., control of crystal form). Additionally, recent rulings on the use of alternative, commercially viable solid forms not protected by patents from

innovator companies have opened the market to generic competition [68–79]. In order to mitigate these risks, and to save time and reduce costs, many pharmaceutical companies have begun to re-evaluate their strategies for solid form screening and are looking to HT crystallization technologies to address the needs for more rapid and comprehensive exploration. In this section, the application of HT crystallization to highly polymorphic systems is reviewed, including specific cases of compounds exhibiting latent polymorphism.

Polymorphic systems are quite common among many types of organic crystals [7]. For the purposes of this review, compounds exhibiting more than three polymorphic forms will be classified as being "highly polymorphic". While only a handful of well-known organic compounds are considered for practical purposes to be non-polymorphic, e.g., aspirin [80,81], sucrose and naphthalene [7], it should be stressed that one will never be able to exclude the possibility of polymorphs appearing, even a century after the initial discovery of the compound. So far, no polymorphs of aspirin have been found, despite the proposal by Payne et al. [80] that polymorphic forms may exist. In contrast, acetaminophen form III was observed by Burger in 1982 using thermal microscopy [82], but it took another 20 years for a crystal structure to be proposed [40]. Many reports exist on the polymorphic nature of specific drug compounds with one or two alternative packing modes for the same chemical composition. However, literature examples of compounds with more than three packing modes are considerably rarer, as will be summarized shortly. It should be noted that the increased number of reports on highly polymorphic compounds in recent years is likely the result of enhanced screening practices and more sensitive characterization techniques.

Highly polymorphic compounds present several challenges in drug development. First, the generation of different forms is often not a simultaneous event, but rather a gradual evolution of form diversity leading to the branding of a compound as being highly polymorphic. Consequently, once more than one form is identified, concern is raised that additional forms may eventually be discovered. For instance, the 13 polymorphs of phenobarbitone evolved over ca. 13 years [7], and a fourth polymorph of carbamazepine was reported in 2002, a full two decades after the

publication of the structures of the initial three forms [83]. Second, selection of the preferred form of a highly polymorphic compound for development demands a complex set of thermodynamic and kinetic investigations, due to the geometric increase in the number of stability relationships that need to be established. More complexity arises when some polymorphic pairs are enantiotropic, exhibiting a switch in the identity of the stable form as a function of temperature. Third, concerns over bio-performance and the impact of a large number of polymorphs on processing lead to regulatory issues that need to be addressed. Decision trees [58] have been established to aid scientists in assessing the impact of polymorphic change and have been incorporated into the ICH guidelines [84]. Lastly, the analytical challenge of monitoring polymorph content in the dosage form increases as the number of possible forms grows, particularly with low dose compounds where the concentration of drug in the formulation is small.

The literature on highly polymorphic pharmaceuticals is relatively sparse, but several examples of compounds known to have four or more polymorphic forms are available in the literature and are summarized in Table 2. In addition to these drug examples, the pharmaceutical ingredients mannitol and aspartame have been shown to exhibit 4 and 5 polymorphs, respectively [7]. The phenomenon in inactive exci-

Table 2
Examples of highly polymorphic drug compounds in the literature

Compound	Number of reported polymorphs	Other forms	Reference(s)
Phenobarbitone	13		[7,p.255]
Cimetidine	7	Hydrates	[7,p.73]
'ROY'	7	7th form found after the initial publication	[111,112]
Sulfathiazole	5	Numerous solvates	[113]
Carbamazepine	4	Dihydrate and numerous solvates	[28,45,83,85]
MK-996	9	Hydrate	[87]
MK-A	4	2 hydrates and numerous solvates	[86]

pients may well be under-appreciated due to lack of study.

In general, pharmaceutical polymorphism is likely to be underreported in the literature, since much of the polymorphism research is carried out in companies. As a result of growing interest in the subject and advances in techniques to study polymorphism, it is expected that reports of extreme form diversity will grow. Conferences on the subject, such as the ACS ProSpectives symposium, reflect the appreciation for the complexities introduced by the appearance of polymorphism in important materials such as pharmaceuticals. Work has recently commenced to understand the opportunities and challenges of using HT technologies in pursuit of rapid identification and characterization of the large number of forms presented by highly polymorphic compounds. Three published case studies and two examples that are in press at the time of this review will be highlighted.

Form IV of carbamazepine was reportedly discovered as the result of crystallization trials in the presence of hydroxypropyl cellulose HPC [83]. Subsequent to this publication, Lang et al. [28] published the use of polymers to influence polymorphic form using a 96-well plate system for the screening of polymorphs of carbamazepine and acetaminophen. In all, 84 different polymers were employed to direct nucleation. Form IV of carbamazepine was found to crystallize from methanol in the presence of hydroxypropyl cellulose, poly(4-methylpentene), poly(*R*-methylstyrene) or poly(*p*-phenylene ether-sulfone). Using the same approach, the monoclinic and orthorhombic forms I and II, respectively, of acetaminophen were also isolated. While observation of metastable form III was not reported in this study, the strategy of employing polymeric additives is of interest, as it can direct the course of crystallization and because polymeric impurities may be in contact with a drug substance and/or formulation at various points in development.

Another approach, reported by Anquetil et al. [85], identified selective conditions for the crystallization of carbamazepine polymorphs forms I and III, as well as the dihydrate, from methanol and/or methanol/water solutions by thermal processing in a microliter cell format (i.e., 35–100 μl). Optical laser trapping was used in situ to target the microcrystals for real-time form analysis using Raman spectroscopy. The crystal-

lization process was monitored optically and with Raman spectroscopy as a function of temperature and time. The study revealed the conversion of form I to form III, as evidenced by a change in characteristic crystal habit from needles to prisms. Raman spectroscopy on the solution phase measured the saturation solubility of each crystal form produced. Although only several experiments were carried out in this study, the authors advance the microfluidic cell format as a potentially viable system for HT polymorph screening.

A third report details the use of in situ Raman spectroscopy to optimize process conditions. The compound MK-A has four anhydrous polymorphs and several other forms, including two hydrates and numerous solvates [86]. The study gives an example of the complex thermodynamic relationships (monotropic and enantiotropic pairs) that can exist in highly polymorphic systems and demonstrates the power of in-situ methods for monitoring the crystallization process.

The angiotensin-II antagonist MK-996 is an example of a highly polymorphic compound (Table 2) [87]. The structure of MK-996, depicted in Fig. 6, contains seven rotatable bonds, the conformations of which could lead to many configurations for crystal packing. HT crystallization experiments with MK-996 in 96-well arrays comprising over 1500 discrete recrystallization trials from a set of 21 solvents or solvent mixtures yielded 186 solids, which were harvested over a period of 7 days [87]. PXRD analysis of these solids suggested the presence of at least 18 distinct
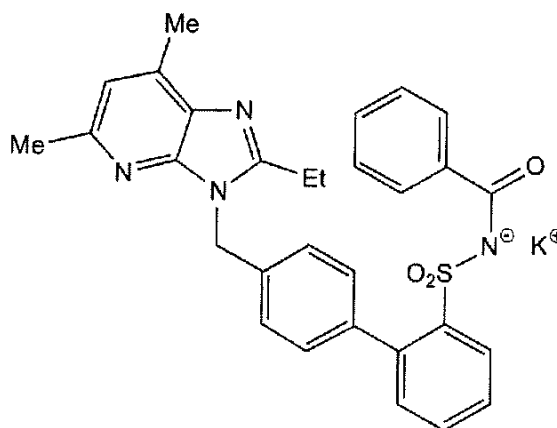
Fig. 6. The molecular structure of the angiotensin-II antagonist MK-996 [87].

forms, some resulting from solvent-mediated recrystallization. A hydrate (originally named form I), obtained by slurry conversion in the presence of aqueous solvent mixtures in the HT experiments, was the form previously selected for pharmaceutical development. Importantly, a form (form D) reported by the innovator [87] to be a "disappearing polymorph" [88] once form I appeared, was also found in the HT screen. Clearly, sufficient experimentation with rationally selected diverse conditions affords the possibility to regenerate elusive forms.

Sertraline HCl, the active ingredient in the antidepressant Zoloft®, is found in various crystal forms. The molecular structure for Sertraline HCl is illustrated in Fig. 7. Information on various solid phases can be found in patent disclosures filed by several companies [89–92]. Survey of these documents, which published between 1992 and 2001, reveals data for 27 purported crystal forms of Sertraline HCl, including 17 polymorphs, 4 solvates, 6 hydrates and the amorphous solid. Further analysis and comparison of characterization data for the various forms presented in the patents revealed that mixtures have been mistaken for real polymorphs on at least two occasions, and at least two polymorphs were disclosed more than once (by different workers each time). In addition, the hydrate forms reported were not readily identified as polymorphic and many of the forms are likely transient, e.g., only identified by variable-temperature and humidity-controlled XRD. With the help of HT crystallization, the extent of true polymorphism of the HCl salt was estimated at eight forms so far [92]. Two new solvates were also found in the HT studies. Care should be taken in isolation of such forms, particularly at small to intermediate scale, as desolvation of solvates due to aggressive drying

during processing may cause one to overlook solvated forms [93]. Comparing the results of the HT study to the congruence of historical data, one can conclude that HT screening gives rise to relevant forms of the drug in a time frame of weeks rather than years. One metastable form, polymorph IV, remained elusive in the hands of the authors [92]. The lack of observation of form IV may be due to a subtle purity difference between early batches at Pfizer and the materials available for testing in the HT screen. Clearly, impurity effects should be explored further [32].

To date, HT studies on highly polymorphic materials highlight the importance of varying processing conditions (including solvent conditions, degree of supersaturation, method of crystallization, desolvation of solvates, inclusion of additives, thermal microscopy, etc.) to find as many forms as possible. It has been shown that multiple process modes, including HT processing, coupled with detailed follow-up characterization studies of form stability, facilitate insight into crystal form diversity [40]. Such a multimode strategy becomes valuable in the quest for the most comprehensive dataset possible for a given pharmaceutical material.

Undoubtedly, the definition of highly polymorphic materials and their frequency will evolve in the age of HT crystallization [40,60] and with the aid of ever improved solid-state analytical capabilities [18,94,95]. The value of employing multiple processing techniques to elucidate as many crystal forms as possible will be demonstrated, as it is expected that no single technique will generate all forms of a given compound. Without doubt, HT crystallization strategies will be used, as a complement to other techniques, to identify issues of polymorphism early, thus allowing drug development scientists to react appropriately to information on form diversity of their compounds.

3.3. Avoiding latent polymorphism

Very few cases of latent polymorphism have been reported in the literature. It is likely that many more instances of the phenomenon have occurred, but unless product development was slowed, product performance was impacted, or generic competition was threatened, a spotlight is not usually cast on the issue. As an example of a public polymorph issue, form 2 of ranitidine hydrochloride was discovered 2–
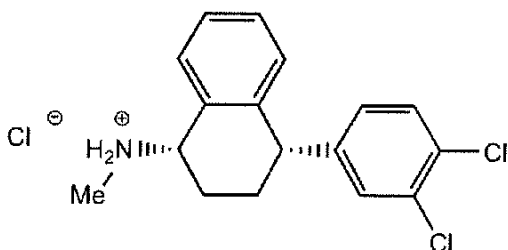

Fig. 7. The molecular structure of the selective serotonin reuptake inhibitor (SSRI) sertraline HCl.

3 years into development but it was (and is) the form still marketed by GlaxoSmithKline [75,76,96]. Paroxetine hydrochloride hemihydrate, the active ingredient in Paxil®, was discovered during development after only an anhydrate had been known for a number of years [97]. The hemihydrate is the form marketed by the innovator, but recent litigations have occurred between the innovator company and generic competition around the anhydrate form.

One of the most recognized cases of latent polymorphism occurred with Abbott Laboratories' Norvir®. Two years after entry into the market, a previously unknown, but thermodynamically more stable, polymorph of the active ingredient (Ritonavir) appeared. This new form (form II) was approximately 50% less soluble in the hydroalcoholic formulation vehicle, resulting in poor dissolution behavior and eventual withdrawal of the original Norvir® capsule from the market [98]. At some considerable cost, a new formulation of Norvir® using form II was eventually developed and launched [99]. In a recent HT crystallization study on Ritonavir, a total of five forms were found: both known polymorphs and three previously unknown forms [99]. The HT polymorph screen, which consisted of 2000 experiments was carried out with less than 2 g of the API and used multiple, and sometimes combined, process methods. The three new forms were described as a metastable polymorph, a crystalline solvate and a non-stoichiometric hydrate. Interestingly, the solvate was easily converted to form I via the hydrate phase using a simple washing procedure, and provided an unusual route to prepare the form I "disappearing polymorph" [88]. Since the crystals of form I prepared using this method retained the small needle morphology of the solvate, the authors suggest that the process may offer a potential strategy for particle size and morphology control. The results of this study emphasize the need for more comprehensive studies of form diversity in the early stages of drug development to avoid risks of form conversion downstream, and highlight the advantage of combining parallel HT crystallization experimentation with detailed physicochemical analyses to identify the diversity of solid forms in which a given molecule can exist. Clearly, late stage discovery of new forms or form conversion can have serious competitive and regulatory implications (e.g., process control), especially in cases where the new forms are not bioequivalent.

3.4. Prediction of crystallization and polymorphism: applications to pharmaceutical form studies

Crystal structure prediction is a challenging area of research. Due to the overwhelming influence of packing forces in determining crystal structure, it remains extremely difficult to predict the structural impact of subtle conformational effects and weak interactions between adjacent molecules in a crystalline arrangement. Although significant progress has been made in the last decade, crystal structures are by and large not reliably predictable from first principles [88]. While this important area of theoretical research is too large a topic to be considered in detail here, a brief overview of the successes and challenges will be presented, and the potential for using HT crystallization as a validation to aid model development will be highlighted. For a more detailed discussion on polymorph and crystal structure prediction, refer to the article by Price [100] in this issue.

Polymorph prediction of pharmaceuticals is thwarted by the complexity of active pharmaceutical molecules. The number of degrees of freedom in torsion angles and the molecule count in the unit cell (which can be deduced by such techniques as solid-state NMR [94]) are frequently too great to allow computations on a reasonable time scale. Additionally, predictions are typically carried out one space group at a time. This limitation is mitigated by the fact that over 90% of the organic compounds in the Cambridge Structural Database (CSD) [101] crystallize in only a few space groups [100]. We know of only one example where predictions have been extended to multicomponent systems [102]. The prevalence of multicomponents systems, some of which have charge transfer (salts) and many of which exist as hydrates, solvates or mixed hydrate/solvates, essentially limits the usefulness of the prediction methods to neutral compounds. Various other technical issues remain as the science of crystal structure prediction matures [100]. Some of these issues were highlighted in two blind tests that were conducted in recent years to determine the accuracy and robustness of crystal structure prediction [103]. In the latest round, 17 methods were used to predict structure, yielding only three correct predictions [104]. For one of the compounds used in the study, experimental characterization of a second, more stable, polymorph provided the key to the correct prediction by three participating

research groups. The structure could have easily been overlooked, leading to the misinterpretation of the results as an apparent failure of the computational methods. Thus, compounds that are amenable to structure prediction are not always studied experimentally to the extent necessary to ensure that the relevant forms have in fact been discovered and characterized ahead of computational studies.

Despite the challenges, a few methods have been developed that allow structure prediction of small, relatively rigid organic compounds with only a few functional groups in several important space groups [17,105,106]. Polymorph Predictor™ has been implemented within the commercial software Cerius2 (C2 Polymorph by Accelrys). In general, current prediction methods generate large ensembles of different packing arrangements along with calculations of relative energetics. In reality, many of the calculated structures are not observed, giving the appearance of over-prediction of polymorphism. This was apparently the case with acetaminophen (paracetamol) [107]. In their study of the drug, Beyer et al. [107] calculated 14 structures, 2 of which were the known monoclinic (stable) and orthorhombic forms. The remaining 12 structures were considered as candidates for the metastable form III, which had been observed by thermal microscopy methods [82] but for which diffraction data were unavailable. Using calculations of mechanical properties and morphology, Beyer et al. separated the 12 energetically feasible structures into two groups, based on the likelihood of each structure to exist as a stable form. Shortly after the publication of the prediction study, the experimental powder pattern of form III became available [40]. Rietveld refinement and comparison of the experimental diffraction results with the theoretical powder patterns published by Beyer et al. yielded a monoclinic structure solution for form III. This structure is in fact part of the prediction set, but was considered an unlikely contender based on its extreme plate-like morphology. The potential for complementarity of HT crystallization and polymorph prediction is evident from these studies. In one sense, polymorph prediction can serve as a yardstick for "risk assessment" when it comes to form diversity, but inevitably one will require experimental data to assess the scope of polymorphism that can be elicited and the precise relative stabilities of different crystalline arrangements.

Opportunities do exist for current use of predictions in solid form discovery. For instance, certain hydrogen-bonding motifs or molecular layer types may be observed in predicted structures. Such information can be used to aid the design of crystallization experiments. It might be desirable to employ a particular type of interaction with salt selection or co-crystal formation by the strategic selection of crystallization conditions, solvents, additives and processing methods [22,23]. In addition, since transient or metastable crystalline species may be difficult to characterize accurately, one may use predicted structures to estimate various physical data. For example, powder diffraction patterns may be used to assist the accurate description of these metastable forms [40]. Continued development of theoretical methods coupled with validation of the predictions by extensive crystallization screening will lead to better models and computational methods. At present, experimental methods must still be relied upon to assess the potential form diversity of a given compound. It will be important to concurrently push the limits on theoretical prediction and HT crystallization, in order to advance our understanding of the nature and extent of polymorphism in pharmaceutical compounds.

3.5. Engineering of co-crystals

Co-crystals of drugs and drug candidates represent a new type of material for pharmaceutical development. They are part of a broader family of multicomponent crystals that also includes salts, solvates, clathrates, inclusion crystals and hydrates as shown in Scheme 2. The primary difference between solvates and co-crystals is the physical state of the isolated pure components: if one component is a liquid at room temperature, the crystals are designated as solvates; if both components are solids at room temperature, the crystals are designated as co-crystals. While at first glance these differences may seem trivial, they have profound impact on preparation, stability and ultimately on the ability to develop products.

In general, it is usually easier to initially prepare solvates than co-crystals, and indeed, solvates are often found as by-products of polymorph and salts screens. Co-crystals have been prepared by melt-crystallization, grinding and recrystallization from solvents [1]. Sol-

Multi-component Crystals
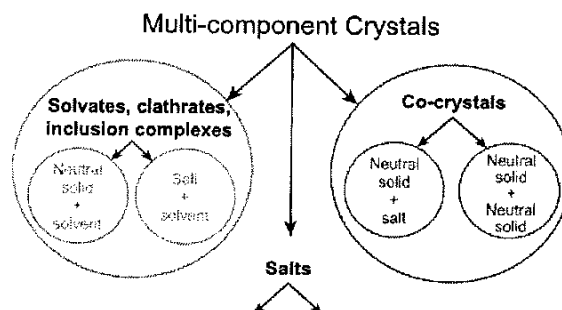
Scheme 2. Types of multicomponent crystals.

vent systems for co-crystals must dissolve all components, but must not interfere with the interactions necessary for co-crystal formation. The need to try many solvent combinations and the availability of multiple co-crystal formers creates a diversity that is ideally suited for exploration by HT systems.

Co-crystals have the potential to be much more useful in pharmaceutical products than solvates or hydrates. The number of pharmaceutically acceptable solvents is very small, and because solvents tend to be more mobile and have higher vapor pressure, it is not unusual to observe dehydration/desolvation in solid dosage forms. Solvent loss frequently leads to amorphous compounds, which are less chemically stable and can crystallize into less soluble forms. In contrast, most co-crystal formers are unlikely to evaporate from solid dosage forms, making phase separation and other physical changes less likely.

Examples of co-crystals have existed in conductive organic crystals, non-linear optical crystals, dyes, photographic materials pigments and agrochemicals for some time [7]. Two recent papers by Fleischman et al. [43, 45] emphasize the importance of understanding "supramolecular synthons" in synthesizing co-crystals containing pharmaceutical agents. For example, the ability to insert 4,4′ -bipyridine between the carboxylic acid dimers of aspirin, *rac*-ibuprofen and *rac*-flurbiprofen was recently reported [43]. The three examples clearly demonstrate the generality of the use of a pyridine-carboxylic acid heterosynthon II

(Scheme 3) to replace a dicarboxylic acid dimer homosynthon I. A second study focused on finding multiple solvates and co-crystals of carbamazepine [45]. Carbamazepine polymorphs crystallize as amide dimers, each of which ties up the polar amide functional groups through homosynthon III. Crystal structures shows that each dimer contains a peripheral H-bond donor and acceptor pair that remain unused due to geometric constraints imposed by the drug molecule. Simple H-bond acceptor solvents like acetone and DMSO insert themselves to fill voids between the adjacent pairs of dimers [45]. Multiple co-crystals formers having hydrogen bond acceptors likewise insert themselves into the void. The homosynthon can also be broken to form heterosynthon IV, an amide-carboxylic acid dimer [45]. This was achieved to form solvates with acetic, formic and butyric acids, and co-crystals with trimesic and nitro-isophthalic acid.

A recent study of adducts of acetaminophen (paracetamol) with ethers and amines provides additional examples of supramolecular synthons for co-crystal formation [108]. While amide-amide homosynthon could have formed, both known forms of the pure material consist of linear head-to-tail chains held together through motif VI; the chains are cross-linked through synthon VII. The linear chain structure is preserved in co-crystals with 4,4′ bipyridine, but the cross-linking interaction VII is replaced by VIII, in which the 4,4′ bipyridine is hydrogen bonded to the amide hydrogen. The chains remain cross-
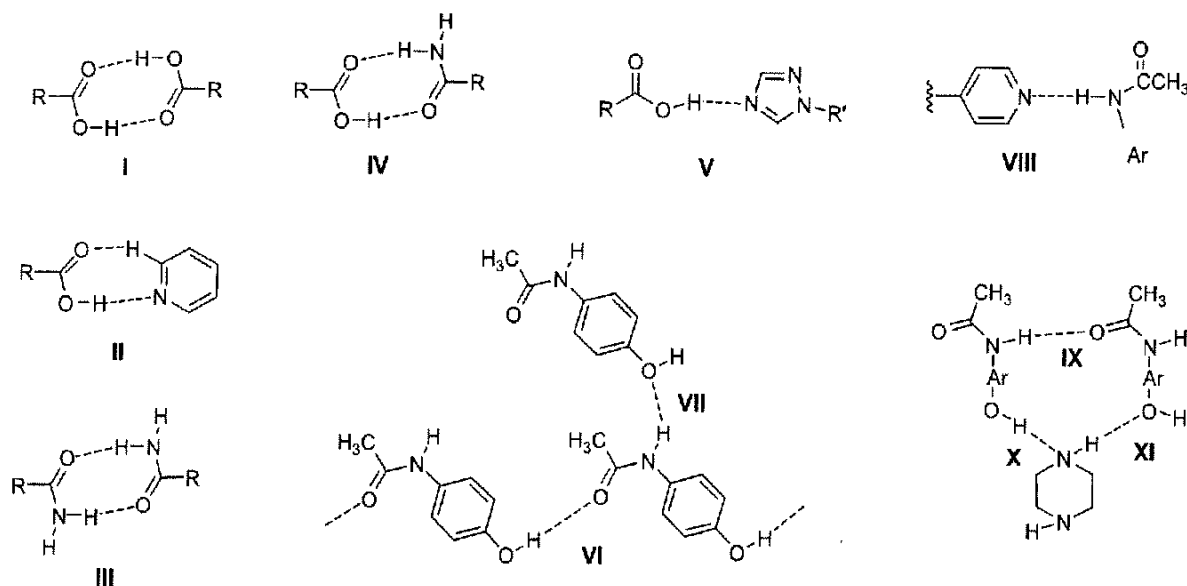
Scheme 3. Supramolecular synthons observed in co-crystals.

linked but only through pi-stacking interactions between 4,4' bipyridine pairs on neighboring chains. In co-crystals with piperazine, the acetaminophen forms head-to-head chains through IX. Each chain is joined to the next through a layer of piperazine molecules that interact through heterosynthons X and XI. The paper also includes many solvates that will not be reviewed here, but their synthons should be applicable to co-crystal formation.

The above studies focused on demonstrating the use of supramolecular synthons to create novel crystalline phases. The variety of structures observed provides hope that some forms will have superior performance in pharmaceutical dosage forms. However, the studies stop short of providing data on the physical properties, such as solubility, necessary to evaluate their utility. Furthermore, only the saccharin and nicotinamide co-crystals of carbamazepine represent pharmaceutically acceptable co-crystals. Crystals containing two drugs may appear to be a good technique for making combination products of two drugs, but unless the two drugs are dosed only in stoichiometric ratios consistent with the co-crystal composition, such crystals would still need to be co-formulated with at least one of the bulk drugs in order to satisfy the clinical requirements.

We recently reported on the discovery and dissolution properties of pharmaceutically acceptable co-crystals consisting of hydrogen-bonded trimers of two molecules of cis-itraconazole and one molecule of a 1,4-dicarboxylic acid resulting from a HT crystallization screen [44]. The crystal structure of the succinic acid co-crystal (Fig. 8) revealed an unanticipated interaction between the triazole of itraconazole and the carboxylic acid (heterosynthon V in Scheme 3). The extended succinic acid molecule fills a pocket, bridging the triazole groups. The interaction between the 1,4-diacid and the strongest base on itraconazole (piperazine) is absent in the co-crystal structure. Other 1,4-diacids including fumaric acid, L-malic acid and L-, D- and DL-tartaric acids also yielded co-crystals with itraconazole, but co-crystals could not be made from maleic acid with Z-regiochemistry, or from 1,3- or 1,5-dicarboxylic acids. Hence, geometric fit appears to be more important than acid-base chemistry in directing crystallization of the compounds of itraconazole with 1,4-dicarboxylic acids.

Identification of multiple crystal forms of the same drug with acceptable solubility, dissolution rate and stability enables selection of the optimal form for dosage form development. To demonstrate this feature, the dissolution of itraconazole co-crystals in
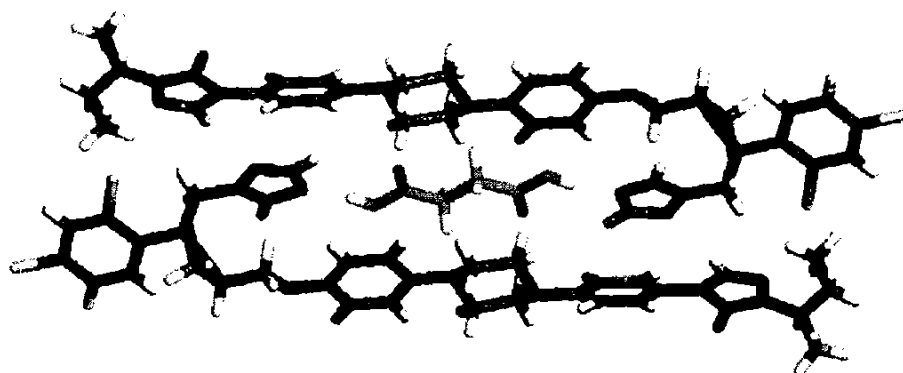
Fig. 8. Trimer unit of the itraconazole succinic acid co-crystal from single crystal X-ray structure (from [44], with permission).

aqueous medium was studied to assess their potential impact on bioavailability of the drug from a solid dosage form. Fig. 9 compares the dissolution profiles of the co-crystals into 0.1 N HCl to those of crystalline itraconazole-free base (95 % of all crystalline particles < 10 μm) and commercial Sporanox® beads (amorphous itraconazole). The malic acid co-crystal rivals the dissolution of the commercial product. In general, the co-crystals behave more similarly to Sporanox® than the crystalline-free base. The co-crystal forms achieve and sustain 4- to 20-fold higher concentrations than that achieved from the crystalline-free base. The practical implication is significant, since the ability to form a supersaturated solution, even transiently, can have dramatic impact on absorption and bioavailability.
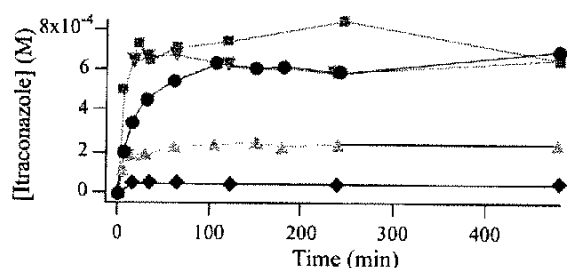

Fig. 9. Dissolution profiles into 0.1 N HCl at 25 °C plotted as itraconazole concentration ([itraconazole]) as a function of time for Sporanox® beads (■), crystalline itraconazole-free base (♦) and co-crystals of itraconazole with L-malic acid (▼), L-tartaric acid (●) and succinic acid (▲) (from [44], with permission).

Co-crystals represent a class of pharmaceutical materials of interest, both in terms of projected diversity and applicability. The study of co-crystals, along with polymorphs, solvates, salts and hydrates, is perfectly suited to HT crystallization experimentation and should be considered part of the form selection processes.

4. Post-screening analyses and form selection

Several functional characteristics must be considered in the selection of a suitable crystal form for a pharmaceutical dosage form. HT crystallization has the potential to create a larger pool of crystal forms for which functional parameters, such as dissolution rate, chemical stability, flow and compressibility, must be determined and compared. Strategies to accomplish ranking of the numerous forms must be devised. An example is the adaptation of HT for solubility measurement. The plot in Fig. 9 illustrates results of a plate-based kinetic dissolution assay in which various forms of a compound were placed in simulated gastric fluid and monitored for dissolution as a function of time. The schematic in Fig. 10 shows how such an analysis can be accomplished in a 96-well filter plate. The concentration at a given time point is determined after filtration of the suspension by quantification using either UV or HPLC with UV detection.

While the entire plate is filtered at one time, different time points can be achieved by timing the addition of dissolution medium such that the aliquot
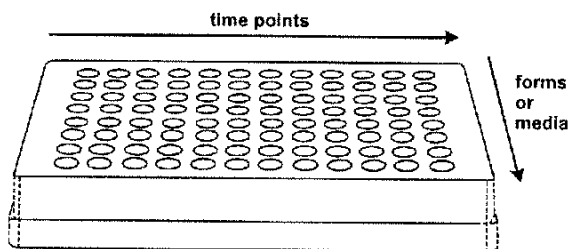
time points

Fig. 10. Schematic of a 96-well dissolution filter plate.

for the longest time point desired is dispensed first and the shortest one comes last. Instead of varying the form along one axis of the plate, one can choose to study the dissolution of a single form into several different media (see Fig. 10). Equilibrium solubility can be determined in a variety of solvents and at different temperatures using a similar principle to the dissolution plate. A demonstration has been provided using automated React-IR analysis [109]. Other functional parameters, such as solid-state stability and thermal properties, can be adapted to HT. Such systems for ranking the stability of forms generated from HT crystallization await publication and review at a future date.

5. Summary and outlook

HT crystallization methodologies are capable of screening hundreds or thousands of crystallization conditions in parallel using small amounts of compound for the identification and characterization of diverse forms of active pharmaceutical ingredients. As demonstrated by numerous case studies from several stages of pharmaceutical development, such technologies have begun to show promise in enabling more comprehensive exploration of solid form diversity. The technologies are likely to provide a landscape of potential operating conditions from which scientists and engineers can design robust and scalable processes for transfer to manufacturing.

The ability to conduct extensive crystallizations with small amounts of material using a variety of solvents, additives and conditions necessarily generates large sets of data. However, the information by itself is of limited value, unless it can be properly analyzed. In order to extract maximum knowledge

from the studies, it is essential to have the ability to design experiments, track samples in the process, collect the data in a relational database, and mine the information using statistical techniques and models in property space that assist the scientist to maximize the value of the data. Such models attempt to fit an output variable to physical properties or descriptors using techniques similar to those used in traditional quantitative structure activity relationships (QSAR). These models can be *carefully* extended to mixtures containing compounds that were not included in the original experiments if validation suggests that the models are sufficiently stable. Significant models that are found in the analysis of the data can be stored in the database for later retrieval and use to direct iterative experiments. The power of this approach becomes increasingly more visible when several properties are being co-optimized, as can be very important in the pharmaceutical development process where such properties as oral bioavailability, stability and processability need to be reconciled. The availability of a map of conditions that lead to the formation of different forms (salts, hydrates, solvates, polymorphs, co-crystals) of the drug can be valuable to the process chemists or engineers as they develop scalable processes to produce materials suitable for development and registration.

For many years, the value of composition of matter (CoM) patents on new chemical entities, including where appropriate, pharmaceutically acceptable salts, has been well appreciated. However, it is only within the last decade or so that the application of CoM patents has been significantly extended to cover all forms of the compound, including hydrates, solvates, co-crystals and polymorphs. Unlike salts, which for the most part can be prophetically claimed based on an understanding of the chemical structure of the compound and its ionization constants, the existence and identity of hydrates, solvates, co-crystals and polymorphs have defied prediction. Therefore, in order to obtain patent protection on these forms, some of which may have significantly different properties and relevance as development candidates, it is essential to prepare them, identify conditions for making them and evaluate their properties as valuable new pharmaceutical materials.

In general, discrete crystal forms are considered non-obvious and patentable. Given the diversity and greater complexity of chemical structures of today's

drug candidates [110], coupled with the advanced technology to identify novel forms, it is common to find multiple forms of drugs [61], some similar, some dramatically different in terms of their in vivo performance. These forms are all candidates for separate intellectual property protection. Therefore, it is incumbent on the innovator of a new drug candidate to identify and patent these forms in order to optimally protect their investment in the compound. Recent case studies suggest that identifying and patenting all forms of new chemical entities should be a primary strategy of all innovators of novel drugs. In this regard, the use of HT crystallization technologies for rapid, comprehensive discovery and characterization of solids form diversity offers significant advantages for the development of a strong intellectual property position.

With the advent of HT crystallization methods, appreciation for the landscape of physical form for drug development has begun to change. Use of these systems has the potential to facilitate drug development by saving valuable time in selecting the optimal physical or chemical form of a given compound. HT systems that generate rich datasets offer the ability to develop a more fundamental understanding of the crystallization process, based on knowledge generated from large numbers of experiments on diverse compounds. Having such information at an early stage minimizes the risk of process modifications resulting in form changes and provides the opportunity to gain more comprehensive intellectual property coverage. In addition, comprehensive form data help address important regulatory questions related to the number of solid forms of an API and the relationships between them.

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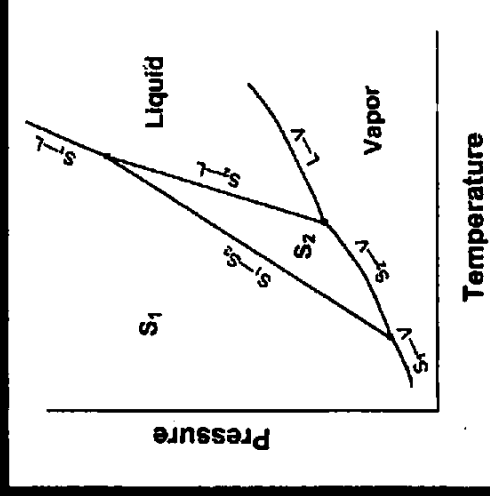
5

Generation of Polymorphs, Hydrates, Solvates, and Amorphous Solids

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I. METHODS EMPLOYED TO OBTAIN UNIQUE POLYMORPHIC FORMS

Organic medicinal agents that can exist in two or more solid phases often can provide some distinct advantages in particular applications. The metastable solid may be preferred in those instances where absorption of the drug is dissolution rate dependent. The stable phase may be less susceptible to chemical decomposition and may be the only form that can be used in suspension formulations. Often a metastable polymorph can be used in capsules or for tableting, and the thermodynamically stable form for suspensions. Factors related to processing, such as powder flow characteristics, compressibility, filterability, or hygroscopicity, may dictate the use of one polymorph in preference to another. In other cases, a particular form may be selected because of the high reproducibility associated with its isolation in the synthetic procedure.

It is essential to ascertain whether the crystalline material that results from a synthetic procedure is thermodynamically stable before conducting pivotal trials, since a more stable form may be obtained subsequently, and it may be impossible to produce the metastable form in future syntheses. Conversion from one polymorph to another can occur during processing or upon storage. An additional incentive for

isolating and identifying polymorphs that provides certain advantages is the availability of subsidiary patents for desirable polymorphic forms, or for retaining a competitive edge through unpublished knowledge. In 1990 Byrn and Pfeiffer found more than 350 patents on crystal forms granted on the basis of an advantage in terms of stability, formulation, solubility, bioavailability, ease of purification, preparation or synthesis, hygroscopicity, recovery, or prevention of precipitation [1].

One question that is likely to arise during the registration process is "What assurance can be provided that no other crystalline forms of this compound exist?" It is incumbent on the manufacturer of a new drug substance to show that due diligence has been employed to isolate and characterize the various solid-state forms of a new chemical entity. This may seem to be a daunting task, particularly in light of the widely quoted statement by Walter C. McCrone [2] that "Those who study polymorphism are rapidly reaching the conclusion that all compounds, organic and inorganic, can crystallize in different crystal forms or polymorphs. In fact, the more diligently any system is studied the larger the number of polymorphs discovered." On the other hand, one can take comfort from the fact that some important pharmaceuticals have been in use for many years and have, at least until now, exhibited only one stable form. Indeed, it seems to this author that there must be particular bonding arrangements of some molecules that are so favorable energetically as to make alternate arrangements unstable or nonisolatable.

In the future, computer programs using force-field optimization should be perfected to the point where it will be possible to predict, with confidence, that a particular crystalline packing arrangement is the most stable that is likely to be found. These programs also may make it possible to predict how many alternate arrangements having somewhat higher energy can potentially be isolated [3,4]. Until that time, the developmental scientist is handicapped in attempting to predict how many solid forms of a drug are likely to be found. The situation is further complicated by the phenomenon of "disappearing polymorphs" [5], or metastable crystal forms that seem to disappear in favor of more stable ones.

Some polymorphs can be detected, but not isolated. Hot stage microscopy has been used extensively to study polymorphic transfor-

mations. The microscopist can detect numerous polymorphic transformations, but the individual polymorphs often prove to be so unstable that they cannot be isolated by the usual methods. An excellent example of this is the work of Grießer and Burger on etofylline [6]. These authors identified five polymorphic forms by thermomicroscopy, but only stable Modification I could be obtained by recrystallization, even when seed crystals from the hot stage were used. Similarly, Kuhnert-Brandstätter, Burger, and Völlenklee [7] described six polymorphic forms of piracetam, only three of which could be obtained by solvent crystallization. All the others were found only by crystallization from the melt. What, then, is a careful investigator to do?

In this chapter, the various methods used to isolate polymorphs, hydrates, and solvates will be described. As Bernstein [8] has observed, "The conditions under which different polymorphs are obtained exclusively or together also can provide very useful information about the relative stability of different phases and the methods and techniques that might be necessary to obtain similar structures of different chemical systems." In this context, it is hoped that the following information will prove useful in devising a "screening" protocol for the preparation of the various solid state forms of pharmaceuticals. While one cannot be absolutely certain that no additional forms will be identified in the future, this approach should provide some assurance that "due diligence" has been exercised to isolate and identify crystalline forms that are likely to arise during the normal course of drug development and storage.

A. Sublimation

On heating, approximately two-thirds of all organic compounds are converted partially from the solid to the gaseous state and back to solid, i.e., they sublime [9]. While strictly speaking the term sublimation refers only to the phase change from solid to vapor without the intervention of the liquid phase, it is often found that crystals are formed on cooler surfaces in close proximity to the melt of organic compounds when no crystals were formed at temperatures below the melting point. The most comprehensive information concerning sublimation temperatures of compounds of pharmaceutical interest can be found in tables

in the textbook of Kuhnert-Brandstätter [9]. While the information in these tables is designed primarily for the microscopic examination of compounds, it is also possible to utilize it to determine which compounds might be susceptible to the application of techniques (such as vacuum sublimation) that can be carried out on larger scales and at lower temperatures.

The sublimation temperature and the distance of the collecting surface from the material undergoing sublimation have a great influence on the form and size of the crystals produced. The occurrence of polymorphic modifications depends on the temperature of sublimation. In general, it may be assumed that unstable crystals form preferentially at lower temperatures, while at higher temperatures stable forms are to be expected. Nevertheless, mixtures consisting of several modifications are frequently found together. This is the case for barbital and for estradiol benzoate. It should be obvious that the sublimation technique is applicable only to those compounds that are thermally stable.

A simple test can be used to determine if a material sublimes. A small quantity (10–20 mg) of the solid is placed in a petri dish that is covered with an inverted watch glass. The petri dish is heated gently on a hot plate and the watch glass is observed to determine if crystals are growing on it. According to McCrone [2], one of the best methods for obtaining a good sublimate is to spread the material thinly over a portion of a half-slide, cover with a large cover glass, and heat slowly using a Kofler block. When the sublimate is well formed, the cover glass is removed to a clean slide for examination. It is also possible to form good crystals by sublimation from one microscope slide to a second held above it, with the upper slide also being heated so that its temperature is only slightly below that of the lower slide. Cooling of the cover slip by placing drops of various low-boiling solvents on the top surface will cause condensation of the more unstable forms, the lower temperatures leading to the most unstable forms. On a larger scale, a glass cold finger or a commercial sublimator can be employed. Once crystals of various modifications have been obtained, they can be used as seeds for the solution phase crystallization of larger quantities.

Form 1 of 9,10-anthraquinone-2-carboxylic acid was obtained as needle-like crystals upon sublimation at temperatures exceeding 250°C [10]. Fokkens et al. have used sublimation to purify theophylline for

vapor pressure studies [11]. Sakiyama and Imamura found that stable phases of both 1,3-dimethyluracil and malonamide could be prepared by vacuum sublimation [12].

B. Crystallization from a Single Solvent

Slow solvent evaporation is a valuable method for producing crystals. Solutions of the material being crystallized, preferably saturated or nearly so, are filtered to remove most nuclei and then left undisturbed for a reasonable period of time. The rate of evaporation is adjusted by covering the solution with aluminum foil or Parafilm® containing a few small holes. For a solvent to be useful for recrystallization purposes, the solubility of the solute should be on the order of 5–200 mg/mL at room temperature. If the solubility exceeds 200 mg/mL, the viscosity of the solution will be high, and a glassy product is likely to be obtained. A useful preliminary test can be performed on 25–50 mg of sample, adding a few (5–10) drops of solvent. If all the solid dissolves, the solvent will not be useful for recrystallization purposes. Similarly, highly viscous solvents, and those having low vapor pressures (such as glycerol or dimethylsulfoxide) are not usually conducive to efficient crystallization, filtration, and washing operations. The solvents selected for recrystallization should include any with which the compound will come into contact during synthesis, purification, and processing, as well as solvents having a range of boiling points and polarities. Examples of solvents routinely used for such work are listed in Table 1 together with their boiling points.

The process of solution mediated transformation can be considered the result of two separate events, (a) dissolution of the initial phase, and (b) nucleation/growth of the final, stable phase. If crystals do not grow as expected from a saturated solution, the interior of the vessel can be scratched with a glass rod to induce crystallization by distributing nuclei throughout the solution. Alternatively, crystallization may be promoted by adding nuclei, such as seed crystals of the same material. For example, Suzuki showed that the α-form of inosine could be obtained by crystallization from water, whereas isolation of the β-form required that seeds of the β-form be used [13].

If two polymorphs differ in their melting point by 25–50°C, for

Generation of Polymorphs

Table 1 Solvents Often Used in the Preparation of Polymorphs

Solvent	Boiling point (°C)
Dimethylformamide	153
Acetic acid	118
Water	100
1-Propanol	97
2-Propanol	83
Acetonitrile	82
2-Butanone	80
Ethyl acetate	77
Ethanol	78
Isopropyl ether	68
Hexane	69
Methanol	65
Acetone	57
Methylene chloride	40
Diethyl ether	35

monotropic polymorphs the lower melting, more soluble, form will be difficult to crystallize. The smaller the difference between the two melting points, the more easily unstable or metastable forms can be obtained.

A commonly used crystallization method involves controlled temperature change. Slow cooling of a hot, saturated solution can be effective in producing crystals if the compound is more soluble at higher temperatures; alternatively, slow warming can be applied if the compound is less soluble at higher temperatures. Sometimes it is preferable to heat the solution to boiling, filter to remove excess solute, then quench cool using an ice bath or even a dry ice–acetone bath. High boiling solvents can be useful to produce metastable polymorphs. McCrone [2] describes the use of high boiling solvents such as benzyl alcohol or nitrobenzene for recrystallization on a hot stage. Behne et al. [14] showed that when buspirone hydrochloride is crystallized above 95°C the higher melting form is obtained; below 95°C the lower

melting form is obtained. Thus the lower melting polymorph could be converted to the higher melting polymorph by recrystallizing from xylene (boiling point 137–140°C).

To understand how temperature influences the composition of crystals that form, it is useful to examine typical solubility–temperature diagrams for substances exhibiting monotropic and enantiotropic behavior [15]. In Fig. 1a, Form II, having the lower solubility, is more stable than Form I. These two noninterchangeable polymorphs are monotropic over the entire temperature range shown. For indomethacin, such a relationship exists between Forms I and II, and between Forms II and III.

In Fig. 1b, Form II is stable at temperatures below the transition temperature T_t, and Form I is stable above T_t. At the transition temperature the two forms have the same solubility, and reversible transformation between enantiotropic Forms I and II can be achieved by temperature manipulation. The relative solubility of two polymorphs is a
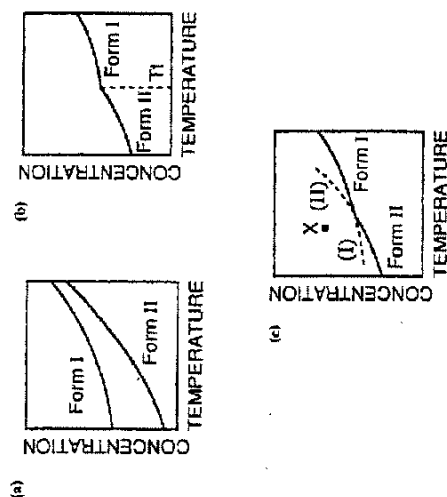

Fig. 1 Solubility curves exhibiting (a) monotropy, (b) enantiotropy, and (c) enantiotropy with metastable phases. (Reprinted with permission of the copyright holder [15].)

convenient measure of their relative free energies. The polymorph having the lower solubility is the more thermodynamically stable form, i.e., the form with the lower free energy at the temperature of the solubility measurement. At room temperature, carbamazepine Form I (m.p. 189°C) is more soluble than is Form III (m.p. 174°C), so the form with the higher melting point is more soluble. The polymorphs are enantiotropic with respect to each other [16].

There are situations in which kinetic factors can for a time override thermodynamic considerations. Figure 1c depicts the intervention of metastable phases (the broken line extensions to the two solubility curves). If a solution of composition and temperature represented by point X (supersaturated with respect to both I and II) is allowed to crystallize, it would not be unusual if the metastable Form I crystallized out first even though the temperature would suggest that Form II would be the more stable (i.e., less soluble) form. This is an extension of Ostwald's law of stages [17], which states that "when leaving an unstable state, a system does not seek out the most stable state, rather the nearest metastable state which can be reached with loss of free energy." This form then transforms to the next most soluble form through a process of dissolution and crystallization. Crystallization of Form I when Form II is more stable would be expected if Form I had the faster nucleation and/or crystal growth rate. However, if the crystals of Form I were kept in contact with the mother liquor, transformation could occur as the more soluble Form I crystals dissolve and the less soluble Form II crystals nucleate and grow. For crystals that exhibit this type of behavior, it is important to isolate the metastable crystals from the solvent by rapid filtration so that phase transformation will not occur.

In the general case, if there are any other polymorphic forms with solubilities below that of Form II, the above-described process will continue between each successive pair of forms until the system finally contains only the most stable (the least soluble) form. The implication of this hypothesis is that, by controlling supersaturation and by harvesting crystals at an appropriate time, it should be possible to isolate the different polymorphic forms. Furthermore, the theory predicts that at equilibrium the product of any crystallization experiment must be the stable form, regardless of the solvent system. It is apparent, however,

from the literature that for some solutes it is the choice of solvent rather than the effects of supersaturation that determines the form that crystallizes [18].

Crystallization of mannitol as a single solute was found to be influenced by both the initial mannitol concentration and by the rate of freezing [19]. In the range of 2.5% to 15%, the δ-polymorph is favored by higher concentrations, whereas the β-polymorph is favored at lower concentrations. At constant mannitol concentration (10%), the α-polymorph is favored by a slow freezing rate, whereas the δ-polymorph is favored by a fast freezing rate.

Kaneko et al. [20] observed that both the cooling rate and the initial concentration of stearic acid in n-hexane solutions influenced the proportion of polymorphs A, B, C, and E that could be isolated. Garti et al. [21] reported that for stearic acid polymorphs crystallized from various organic solvents, a correlation was observed between the polymorph isolated and the extent of solvent–solute interaction.

The reason for using crystallization solvents having varying polarities is that molecules in solution often tend to form different types of hydrogen-bonded aggregates, and that these aggregate precursors are related to the crystal structures that develop in the supersaturated solution [22]. Crystal structure analysis of acetanilide shows that a hydrogen-bonded chain of molecules is aligned along the needle axis of the crystals. This pattern is characteristic of secondary amides that crystallize in a trans conformation so that the carbonyl acceptor group and the –NH hydrogen bond donor are anti to one another. The morphology of acetanilide crystals can be controlled by choosing solvents that promote or inhibit the formation of this hydrogen-bond chain. Hydrophobic solvents such as benzene and carbon tetrachloride will not participate in hydrogen-bond formation, so they will induce the formation of rapidly growing chains of hydrogen-bonded amides. Crystals grown by evaporation methods from benzene or carbon tetrachloride are long needles. Solvents that are proton donors or proton acceptors inhibit chain formation by competing with amide molecules for hydrogen-bonding sites. Thus acetone inhibits chain growth at the –NH end, and methanol inhibits chain growth at the carbonyl end of the chain. Both solvents encourage the formation of rod-like acetanilide crystals, while

mixtures of benzene and acetone give hybrid crystals that are rod-shaped, with fine needles growing on the ends [23].

Some solvents favor the crystallization of a particular form or forms because they selectively adsorb to certain faces of some polymorphs, thereby either inhibiting their nucleation or retarding their growth to the advantage of others. Among the factors affecting the types of crystal formed are (a) the solvent composition or polarity, (b) the concentration or degree of supersaturation, (c) the temperature, including cooling rate and the cooling profile, (d) additives, (e) the presence of seeds, (f) pH, especially for salt crystallization, and (g) agitation [22].

Martínez-Ohárriz et al. [24] found that Form III of diflunisal is obtained from polar solvents, whereas Forms I and IV are obtained from nonpolar solvents. Likewise, Wu et al. [25] observed that when moricizine hydrochloride is recrystallized from relatively polar solvents (ethanol, acetone, and acetonitrile), Form I is obtained, whereas nonpolar solvents (methylene chloride or methylene chloride/ethyl acetate) yield Form II.

In determining what solvents to use for crystallization, one should be careful to select those likely to be encountered during formulation and processing. Typically these are water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, and hexane. Matsuda employed 27 organic solvents to prepare two polymorphs and six solvates of piretanide [26].

According to McCrone [27], in a poor solvent the rate of transformation of a metastable to a more stable polymorph is slower. Hence a metastable form once crystallized can be isolated and dried before it is converted to a more stable phase by solution phase mediated transformation. In some systems the metastable form is extremely unstable and may be prepared only with more extreme supercooling. This is usually performed on a very small scale with high boiling liquids so that a saturated solution at a high temperature that is suddenly cooled to room temperature will achieve a high degree of supersaturation [28].

There are many examples in the literature of the use of single solvents as crystallization screens. Slow crystallization from acetone, acetonitrile, alcohols, or mixtures of solvents yields the Form A of

fosinopril sodium, but rapid drying of a solution of this compound yields Form B, sometimes contaminated with a small amount of Form A [29]. A rotary evaporator can be used to maintain a solution at the appropriate temperature as solvent is being removed.

Form I of dehydroepiandrosterone was obtained by recrystallization from warm ethyl acetate, acetone, acetonitrile, or 2-propanol. Form II was obtained by rapid evaporation, using a vacuum from solutions in dioxane, tetrahydrofuran, or chloroform (which are higher boiling, less polar solvents) [30].

C. Evaporation from a Binary Mixture of Solvents

If single-solvent solutions do not yield the desired phase, mixtures of solvents can be tried. Multicomponent solvent evaporation methods depend on the difference in the solubility of the solute in various solvents. In this approach, a second solvent in which the solute is sparingly soluble is added to a saturated solution of the compound in a good solvent. Often a solvent system is selected in which the solute is more soluble in the component with the higher vapor pressure. As the solution evaporates, the volume of the solution is reduced and, because the solvents evaporate at different rates, the composition of the solvent mixture changes.

Occasionally, crystals are obtained by heating the solid in one solvent and then pouring the solution into another solvent or over cracked ice. Otsuka et al. [31] obtained phenobarbital Form B by adding dropwise a saturated solution of the compound in methanol to water at room temperature. Form E was obtained by the same technique, but by using a saturated solution of phenobarbital in dioxane.

Kitamura et al. have shown that the fraction of Form A of L-histidine decreases quickly when the volume fraction of ethanol in an ethanol–water solvent system increases above 0.2, and that pure Form B is obtained at a 0.4 volume fraction of ethanol [32]. The transformation rate for conversion of Form B to Form A decreases with ethanol concentration. The authors postulated that the concentration of the conformer that corresponds to Form A decreases more with ethanol concentration than that of Form B, and so the growth rate of Form A will also decrease.

An example of precipitation in the presence of a second solvent is seen in the case of indomethacin. The γ-crystal form of indomethacin can be obtained by recrystallization from ethyl ether at room temperature, but the α-form is prepared by dissolution in methanol and precipitation with water at room temperature [33]. Precipitation can also result from the addition of a less polar solvent. Form II of midodrine hydrochloride, metastable with respect to Form I, can be prepared by precipitation from a methanolic solution by means of a less polar solvent such as ethyl acetate or dichloromethane [34].

In Fig. 2, three crystalline modifications of thalidomide are illustrated. These were obtained by solvent recrystallization techniques and differ both in crystal habit and in crystal structure. Two of the forms were obtained from a single solvent, and one from a binary mixture.

D. Vapor Diffusion

In the vapor diffusion method, a solution of the solute in a good solvent is placed in a small, open container that is then stored in a larger vessel containing a small amount of a miscible, volatile nonsolvent. The larger vessel (often a desiccator) is then tightly closed. As solvent equilibrium is approached, the nonsolvent diffuses through the vapor phase into the solution, and saturation or supersaturation is achieved. The solubility of the compound in a precipitant used in a two-solvent crystallization method such as vapor diffusion should be as low as possible (much less than 1 mg/mL), and the precipitant (the solvent in which the compound is poorly soluble) should be miscible with the solvent and the saturated solution. The most frequent application of this technique is in the preparation of single crystals for crystallographic analysis. An illustration of the technique is provided in Fig. 3 [35].

E. Thermal Treatment

Frequently when using differential scanning calorimetry as an analysis technique, one can observe an endothermic peak corresponding to a phase transition, followed by a second endothermic peak corresponding to melting. Sometimes there is an exothermic peak between the two endotherms, representing a crystallization step. In these cases it is often
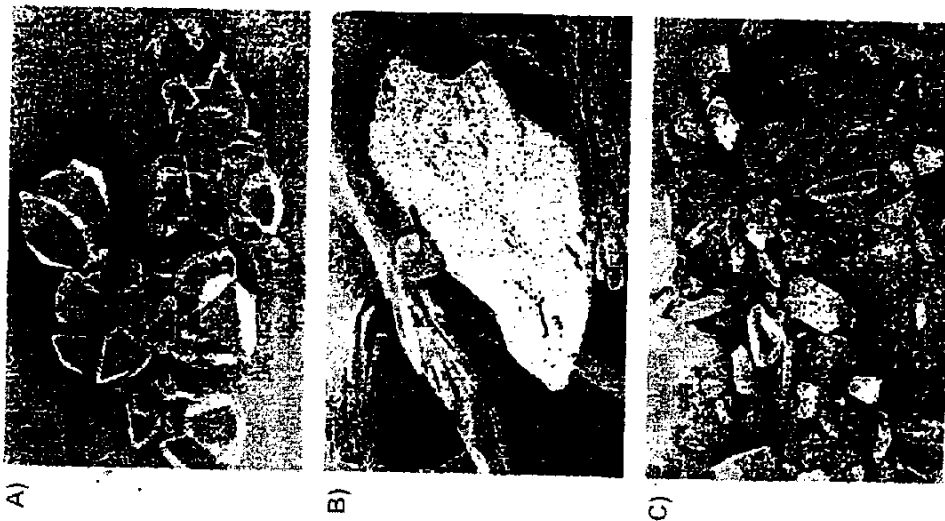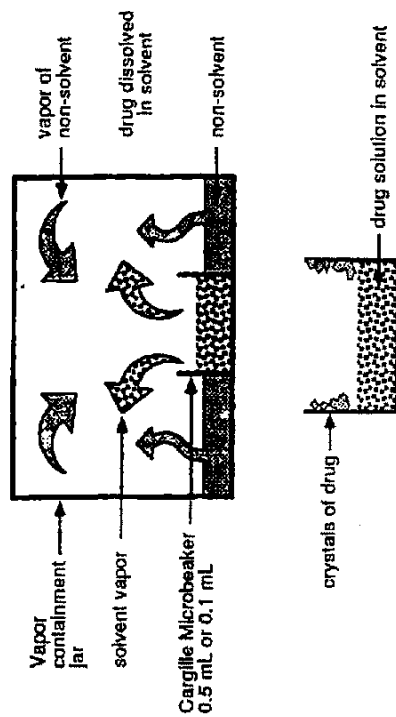
Generation of Polymorphs

Fig. 3 Crystallization by vapor diffusion. (Reproduced with permission of the author [35] and the copyright holder, Pfizer, Inc.)

possible to prepare the higher melting polymorph by thermal treatment. Thus chlorpropamide Form A is obtained by recrystallization from ethanol solution, but Form C is obtained by heating Form A in an oven maintained at 100°C for 3 hours [36]. While the β-form of tegafur is obtained by the evaporation of a saturated methanol solution, the γ-form is obtained by heating the β-form at 130°C for one hour [37]. Form II of caffeine is prepared by recrystallization from distilled water, but Form I is prepared by heating Form II at 180°C for 10 hours [38].

F. Crystallization from the Melt

In accordance with Ostwald's rule [17], the cooling of melts of polymorphic substances often first yields the least stable modification, which subsequently rearranges into the stable modification in stages. Since the metastable form will have the lower melting point, it follows that supercooling is necessary to crystallize it from the melt. After melting, the system must be supercooled below the melting point of the metastable form, while at the same time the crystallization of the more stable form or forms must be prevented. Quench cooling a melt can

Fig. 2 Three crystalline modifications of thalidomide obtained by solvent recrystallization. (A) Form 1 obtained as bipyramids by slow crystallization of thalidomide in 1:1 dimethylformamide:ethanol at room temperature. (B) Form II obtained by immersing a saturated solution of thalidomide in acetonitrile in an ice bath. (C) Form III prepared as tabular crystals from a solution in boiling 1,4-dioxane, filtered, then allowed to cool to room temperature. (Photomicrographs courtesy of Dr. S. A. Botha, the University of Iowa.)

and stored *in vacuo* (380 torr) for 12 hours at 35°C. Similarly, Form III of hydrochlorothiazide was precipitated from sodium hydroxide aqueous solution by the addition of hydrochloric acid [44].

When piretanide was dissolved in 0.1 N NaOH at room temperature and acid was added in a 1:1 ratio (to pH 3.3), piretanide Form C precipitated. However, when the base:acid ratio used was 1:0.95, a mixture of amorphous piretanide and Form C precipitated [45].

H. Thermal Desolvation of Crystalline Solvates

The term "desolvated solvates" has been applied to compounds that were originally crystallized as solvates but from which the solvent has been removed (generally by vaporization induced by heat and vacuum). Frequently, these "desolvated solvates" retain the crystal structure of the original solvate form and exhibit relatively small changes in lattice parameters. For this reason, these types have been referred to as pseudopolymorphic solvates. However, in instances where the solvent serves to stabilize the lattice, the process of desolvation may produce a change in lattice parameters, resulting in the formation of either a new crystal form or an amorphous form. These solvates have been referred to as polymorphic solvates. Byrn [46] has characterized the desolvation of polymorphic solvates as occurring in four steps, (a) molecular loosening, (b) breaking of the host–solvent hydrogen bonds (or other associations), (c) solid solution formation, and (d) separation of the product phase.

The process of desolvating pseudopolymorphic solvates is simpler, involving only the two steps of (a) molecular loosening and (b) breaking of host–solvent hydrogen bonds or associations. Byrn [46] has summarized the desolvation studies performed on caffeine hydrate, theophylline hydrate, thymine hydrate, cytosine hydrate, dihydrophenylalanine hydrate, dialuric acid hydrate, cycloserine hydrate, erythromycin hydrate, fenoprofen hydrate, manganous formate dehydrate, bis(salicylaldehyde) ethylenediamine cobalt (II) chloroformate, cephatoglycine hydrates and solvates, and cephalexin solvates and hydrates. Among factors that influence the desolvation reaction are the appearance of defects, the size of tunnels in the crystal packing arrange-

sometimes result in formation of an amorphous solid that on subsequent heating undergoes a glass transition followed by crystallization [39].

On a somewhat larger scale, one can use a vacuum drying pistol and a high boiling liquid such as chlorobenzene to achieve the desired end. Form II of p-(1R,3S)-3-thioanisoyl-1,2,2-trimethylcyclopentane carboxylic acid was obtained by recrystallization from a 50:50 v/v benzene:petroleum ether mixture. Form I then was obtained by melting Form II in the vacuum drying pistol [40]. Caffeine Form I is prepared by heating Form II at 180°C for 10 hours [38]. Yoshioka et al. [41] observed that when the amorphous solidified melt of indomethacin was stored at 40°C, it partly crystallized as the thermodynamically stable γ-form. Yet at 50°C, 60°C, and 70°C, mixtures of the α- and the γ-form were obtained. Sulfathiazole Form I is obtained by heating Form III crystals (grown from a dilute ammonium hydroxide solution at room temperature) at 170°C for 30–40 minutes [42].

G. Rapidly Changing Solution pH to Precipitate Acidic or Basic Substances

Many drug substances fall in the category of slightly soluble weak acids, or slightly soluble weak bases, whose salt forms are much more soluble in water. Upon addition of acid to an aqueous solution of a soluble salt of a weak acid, or upon addition of alkali to an aqueous solution of a soluble salt of a weak base, crystals often result. These crystals may be different from those obtained by solvent crystallization of the weak acid or weak base. Nucleation does not necessarily commence as soon as the reactants are mixed, unless the level of supersaturation is high, and the mixing stage may be followed by an appreciable time lag before the first crystals can be detected. Well-formed crystals are more likely to result in these instances than when rapid precipitation occurs.

Form I of the x-ray contrast agent iopanoic acid was prepared [43] by dissolving the acid in 0.1 N NaOH, adjusting the pH to 12.5, bubbling nitrogen into the solution, and adding 0.1 N hydrochloric acid until the pH reached 2.15. The resulting precipitate was vacuum filtered

ment, and the strength of hydrogen bonding between the compound and its solvent of crystallization [46].

Rocco et al. [47] obtained Form II of zanoterone by recrystallization from ethanol and vacuum drying at 45°C. Form III was isolated by desolvating the acetonitrile solvate form at 80°C under vacuum, and this was the form chosen for use in the clinical drug product due to the high reproducibility of its isolation during manufacture. Similarly, Forms I and II of stanozolol were obtained by heating solvates of the compound to 205°C and 130°C, respectively [48].

The benzene solvate of iopanoic acid was prepared by rapidly freezing a warm benzene solution of iopanoic acid in a dry ice–acetone mixture [43]. The solid obtained was permitted to melt at room temperature, yielding crystals of the solvate suspended in benzene. When these were vacuum filtered and stored in vacuo (380 torr) for 12 hours at 70°C, Form II was obtained free of benzene.

Dehydration of hydrates can also lead to the formation of unique crystals. Caffeine Form II was prepared by recrystallizing caffeine from water, drying for 8 days at 30°C, and then heating for 4 hours at 80°C [38]. Chloroquine diphosphate 3 : 1 hydrate was converted to the anhydrous form at temperatures above 188°C [49]. Etoposide Form I (a monohydrate) was found to undergo a dehydration reaction in the temperature range of 85–115°C to yield etoposide Form Ia. This form could be melted at 198°C and transformed to etoposide Form IIa, which itself melted at 198°C and crystallized to still another polymorph, etoposide Form IIa at 206°C. Etoposide Form IIa was found to melt at 269°C and convert to its hydrated form, etoposide Form II, when exposed to the atmosphere at room temperature. This hydrate was also found to undergo a dehydration reaction at 90–120°C to yield etoposide Form IIa [50].

Differential scanning calorimetry (DSC) curves of levofloxacin hemihydrate measured under various conditions showed different thermograms. This behavior was attributed to the dehydration process that resulted in a multiple-phase transition. Dehydration at higher temperatures (above 70°C) gave a sharp endothermic peak in the DSC thermogram due to the melting of the γ-form, and at a lower temperature (50°C) it led to the observation of a sharp endothermic peak due to the

melting of the α-form. In contrast, the thermal behavior of levofloxacin monohydrate was not affected by dehydration [51].

I. Growth in the Presence of Additives

The presence of impurities can have a profound effect on the growth of crystals. Some impurities can inhibit growth completely, and some may enhance growth. Still others may exert a highly selective effect, acting only on certain crystallographic faces and thus modifying the crystal habit. Some impurities can exert an influence at very low concentrations (less than 1 part per million), whereas others need to be present in fairly large amounts to have any effect [15].

Additives can be designed to bind specifically to the surfaces of particular polymorphs and so inhibit their achieving the critical size for nucleation, allowing a desired phase to grow without competition [52]. Lahav and coworkers have shown that additives at levels as low as 0.03% can inhibit nucleation and crystal growth of a stable polymorph, thus favoring the growth of a metastable polymorph [53]. They also showed that it is possible to design crystal nucleation inhibitors to control polymorphism.

Davey et al. found that Form I crystals of terephthalic acid could be obtained by crystallization only in the presence of p-toluic acid [54]. Form II, the more stable polymorph at ambient temperatures, was recovered from a hydrothermal recrystallization experiment.

Ikeda et al. [55] determined that indomethacin can exist in three different crystal forms, denoted α-, β-, and γ-, with the α-form possessing a higher solubility than the γ-form. On recrystallization, crystals of the α-form were the first to be deposited, but these converted gradually to the less soluble γ-form. However, in the presence of hydroxypropyl methylcellulose, conversion from the α-form to the γ-form was inhibited, leading to an increase in the solubility of indomethacin.

While the α-form of glycine normally is obtained by recrystallization from water, 3% of racemic hexafluorovaline leads to the precipitation of the γ-polymorph as trigonal pyramids [56]. This additive was designed to be strongly adsorbed at the four (011) crystal faces of the α-form and to bind at only one pole of the polar crystal, thus leaving

the crystal free to grow at the opposite pole. Since it is bound at the slow growing NH_3^+ end of the polar axis, it does not interfere with the fast growing CO_2^- end.

J. Grinding

Polymorphic transformations have been observed to occur on grinding of certain materials, such as sulfathiazole, barbital, phenylbutazone, cephalexin, chloramphenicol palmitate, indomethacin, and chlorpropamide. Byrn [46] has stated that polymorphic transformations in the solid state require the three steps of (a) molecular loosening (nucleation by separation from the lattice), (b) solid solution formation, and (c) separation of the product (crystallization of the new phase). Depending on the material and the conditions employed, grinding can result in conversion to an amorphous substance. With the exercise of care, different polymorphic forms can be obtained. Otsuka et al. [57] showed that metastable Forms B and C of chloramphenicol palmitate were transformed into stable Form A upon grinding at room temperature. Indomethacin was transformed into a noncrystalline solid during grinding at 4°C, and into metastable Form A by grinding at 30°C. Caffeine Form II is converted into Form I with grinding, and a 95% phase conversion was obtained following 60 hours of grinding time [38].

II. METHODS EMPLOYED TO OBTAIN HYDRATE FORMS

Pharmaceutical solids may come into contact with water during processing steps, such as crystallization, lyophilization, wet granulation, aqueous film-coating, or spray-drying. Moreover, they may be exposed to water during storage in an atmosphere containing water vapor, or in a dosage form consisting of materials that contain water (e.g., excipients) and are capable of transferring it to other ingredients. Water may be adsorbed onto the solid surface and/or may be absorbed in the bulk solid structure. When water is incorporated into the crystal lattice of the compound in stoichiometric proportions, the molecular adduct or adducts formed are referred to as hydrates [58]. More than 90 hydrates

are described in various USP monographs. Hydrates can be prepared by recrystallization from water or from mixed aqueous solvents. They can also result, in some instances, from exposure of crystal solvates (such as methanolates or ethanolates) to an atmosphere containing water vapor.

Crystalline substances often form with water molecules located at specific sites in the crystal lattice, which are held in coordination complexes around lattice cations. This type of water is denoted as water of crystallization and is common for inorganic compounds. For example, nickel sulfate forms a well-defined hexahydrate, where the waters of hydration are bound directly to the Ni(II) ion. Extraneous inclusion of water molecules can occur if a coprecipitated cation carries solvation molecules with it. Water also can be incorporated into random pockets as a result of physical entrapment of the mother liquor. Well-defined multiple hydrate species can also form with organic molecules. For example, raffinose forms a pentahydrate.

Although most hydrates exhibit a whole-number-ratio stoichiometry, an unusual case is the metastable hydrate of caffeine, which contains only 0.8 moles of water per mole of caffeine. Only in a saturated water vapor atmosphere will additional amounts of water be adsorbed at the surface of the 4/5-hydrate to yield a 5/6 hydrate [59].

In some instances, a compound of a given hydration state may crystallize in more than one form, so that the hydrates themselves exhibit polymorphism. One such example is nitrofurantoin, which forms two monohydrates that have distinctly different temperatures and enthalpies of dehydration. The monohydrates have quite different packing arrangements, with Form I possessing a layer structure and Form II exhibiting a herringbone motif. The included water molecules play a major role in stabilizing the crystal structures. Whereas water molecules are contained in isolated cavities in Form II, in Form I they are located in continuous channels, and this apparently facilitates the escape of water when these crystals are heated [60].

Another example of hydrate polymorphism is amiloride hydrochloride [61], which can be obtained in two polymorphic dihydrate forms. These forms are indistinguishable by techniques other than x-ray powder diffraction.

It is interesting that scopolamine hydrobromide has been reported

to exist as the anhydrous form, a "hemihydrate," a sesquihydrate, and a trihydrate [62], while the unit cell parameters and the molecular geometry of these are all the same as those of the hemihydrate. This finding suggests that the "hemihydrate" is actually a partially desolvated sesquihydrate.

Ouabaine is another example of a compound that exhibits many different hydration levels, the most hydrated form being stable at the lowest temperature. Thus the nonahydrate phase of ouabaine is obtained from water at 0–15°C, the octahydrate phase at 15–28°C, and the dihydrate phase at 28–90°C. In addition, ouabaine phases corresponding to 4.5 H_2O, 4 H_2O, and 3 H_2O may be obtained from mixtures of water with other solvents. The anhydrous phase of ouabaine anhydrate is crystallized from ethanol at high temperatures [63].

Typically, hydrates are obtained by recrystallization from water. For example, trazodone hydrochloride tetrahydrate was prepared by dissolving the anhydrate in hot distilled water, allowing the solution to remain at room temperature overnight, and storing the collected crystals at 75% relative humidity and 25°C until they reached constant weight [64].

Hydrates can sometimes be obtained by simply suspending the anhydrous material in water, whereupon a form of Ostwald ripening occurs. For instance, aqueous suspensions of anhydrous metronidazole benzoate are metastable, and storage at temperatures lower then 38°C leads to monohydrate formation accompanied by crystal growth [65]. Sorbitol provides another example of this behavior, where slow cooling of a saturated aqueous solution yields long thin needles of sorbitol hydrate [66]. When suspended in water, anhydrous carbamazepine is transformed to carbamazepine dihydrate [67]. In other instances, hydrates can be obtained from mixed solvent systems. Acemetacin monohydrate can be obtained by slow evaporation from a mixture of acetone and water at room temperature [68].

Simply exposing an anhydrous powder to high relative humidity can often lead to formation of a hydrate. On exposure to a relative humidity of 100%, dexmedetomidine hydrochloride is converted to a monohydrate [69]. Droloxifene citrate is an example of a compound that is not very hygroscopic and yet forms a hydrate. Only after storage of the anhydrous form at 85% relative humidity does some sorption of

water occur. The monohydrate phase can be formed by exposing the anhydrous form to 98% relative humidity for ten days at 24°C [70].

III. METHODS EMPLOYED TO OBTAIN SOLVATE FORMS

Often, when solvents are employed in the purification of new drug substances by recrystallization, it is observed that the isolated crystals include solvent molecules, either entrapped within empty spaces in the lattice or interacting via hydrogen bonding or van der Waals force with molecules constituting the crystal lattice. Solvent molecules also can be found in close association with metal ions, completing the coordination sphere of the metal atom. Coordinated solvent molecules are considered as part of the crystallized molecule. A crystal with large empty channels or cavities is not stable because of packing demands. The size and chemical environment of the cavity or channel determine what kind of solvent molecule can be included in the structure and what kind of interaction occurs between solvent and structure.

Depending on the nature of molecular packing arrangements, it may happen that the inclusion of solvent is necessary to build a stable crystal structure. van Geerestein et al. [71] found during numerous crystallization attempts of 11β-[4-(dimethylamino)phenyl]-17β-hydroxy-17α-(1-propynyl) estra-4,9-diene-3-one] that crystals were only obtainable in the presence of n-butyl acetate or n-propyl acetate. The crystal structure of the compound crystallized from n-butyl acetate/methylcyclohexane was solved, and one solvent molecule was found in the crystal structure that showed no strong interactions with the rest of the structure. Apparently, this solvent molecule was necessary to fill empty space resulting after the molecular packing. Solvates in which the solvent fills empty space are generally nonstoichiometric, such as the nonstoichiometric solvates formed by droloxifene citrate with acetonitrile, 2-propanol, ethanol, 1-propanol, and 1-butanol. Typically such solvates exhibit the same x-ray diffraction pattern as does the nonsolvated compound.

When solvent molecules increase the strength of the crystal lattice, they can affect the stability of the compound to solid-state decom-

position. It has been observed that the four solvated and one nonsolvated structures of prenisolone tert-butyl acetate affect the flexibility of the steroid nucleus and the structure-dependent degradation of the compound when exposed to air and light [72].

van der Sluis and Kroon found 1,247 different compounds with cocrystallized solvents in the Cambridge Crystallographic Database [73]. Out of 46,460 total structures, they found 9,464 solvate structures, and 95% of these contained one of the 15 solvents given in Table 2.

The most commonly encountered solvates among pharmaceuticals are those of 1:1 stoichiometry, but occasionally mixed solvate species are encountered. For structures containing more than one solvent type, one generally finds nonpolar solvents crystallizing together on the one hand and polar solvents on the other. For example, the most common solvents found cocrystallizing with water are (in order of im-

Table 2 Distribution of the 15 Most Abundant Solvents in the Cambridge Crystallographic Database, as the Percentage of Solvate Structures

Solvent	Occurrence (%)
Water	61.4
Methylene dichloride	5.9
Benzene	4.7
Methanol	4.1
Acetone	2.8
Chloroform	2.8
Ethanol	2.6
Tetrahydrofuran	2.3
Toluene	2.2
Acetonitrile	1.9
N,N-dimethylformamide	0.9
Diethyl ether	0.9
Pyridine	0.7
Dimethyl sulfoxide	0.5
Dioxane	0.5

Source: From Ref. 73. Reproduced with permission of the copyright owner.

portance) ethanol, methanol, and acetone. An interesting example of a structure containing a polar and a nonpolar solvent is the sodium salt of the antibiotic K-41, p-bromobenzoate monohydrate n-hexane solvate [74], which is crystallized from n-hexane saturated with water. Perhaps the best known mixed solvate is doxycycline hyclate: (doxycycline · HCl)$_2$C$_2$H$_6$O · H$_2$O. Triamterene also forms a mixed solvate, containing one N,N-dimethylformamide molecule and one water molecule within the crystal lattice [75].

The techniques used to obtain solvates are generally similar to the solvent methods used to obtain polymorphs, i.e. crystallization from a single solvent, from mixed solvents, or by vapor diffusion. Sometimes, it is possible to exchange one solvent within the crystal structure for another. When one recrystallizes a hydrate from dry methanol, in most cases one is left with either a methanol solvate or an anhydrous, unsolvated form of the compound.

A large number of solvates have been reported, especially for steroids and antibiotics. It has been observed that cortisone acetate and dexamethasone acetate can be crystallized as 10 different solvates. Dirithromycin, a semisynthetic macrolide antibiotic, crystallizes in two anhydrous polymorphic forms and in at least nine stoichiometric solvate forms. Six of the known solvates are isomorphic, having nearly identical x-ray powder diffraction patterns [76]. In addition to the anhydrate and dihydrate, erythromycin also forms solvates with acetone, chloroform, ethanol, n-butanol, and i-propanol [77].

It may be instructive to consider some examples of solvate formation. The compound 5-methoxysulphadiazine forms 1:1 host–guest solvates with dioxane, chloroform, and tetrahydrofuran [78]. These were prepared by heating to boiling a solution of the sulfonamide in the appropriate solvent, followed by slow cooling to obtain large crystals. Spironolactone forms 1:1 solvates with methanol, ethanol, ethyl acetate, and benzene. It also forms a 2:1 spironolactone–acetonitrile solvate [79,80]. The spironolactone solvates were prepared by crystallization in a refrigerator from solutions that were nearly saturated at room temperature.

Another steroid that forms solvates is stanozolol [81]. Solvates having 1:1 stoichiometry were prepared by recrystallization from methanol, ethanol, and 2-propanol, by heating the compound in the

appropriate solvent to 60–70°C and then cooling to 0°C in an ice bath to induce crystallization. The compound also forms a monohydrate and two polymorphs. The polymorphs were prepared by heating the solvates to either 130°C (Form II) or 205°C (Form I).

Mefloquine hydrochloride is an interesting case of a compound that forms stoichiometric 1:1 solvates on cooling hot (50°C) saturated acetone solutions (Form B, acetone solvate 1:1), hot (50°C) saturated isopropanol (Form I, isopropanol solvate 1:1), and a nonstoichiometric ethanol solvate (2.12% ethanol) from hot (50°C) saturated ethanol, Form E, whose x-ray powder pattern does not change following heating to 80°C, in spite of a decrease in the ethanol level to 0.12%. Mefloquine hydrochloride can also be obtained in a nonsolvated form from hot (70°C) saturated acetonitrile (Form A) and as two hemihydrates from water (Forms D and C) prepared at room temperature and at 30°C [82].

IV. METHODS EMPLOYED TO OBTAIN AMORPHOUS MATERIALS

Solids can exist in crystalline or amorphous form. Crystalline materials have defined structures, stoichiometric compositions, and melting points and are characterized by their chemical, thermal, electrical, optical, and mechanical properties [83]. By contrast, amorphous materials have no clearly defined molecular structure and no long-range order, so their structure can be viewed as being similar to that of a frozen liquid but without the thermal fluctuations observed in the liquid phase. As a result, amorphous materials exhibit the classical diffuse "halo" x-ray powder diffraction pattern rather than the sharp peaks observed in the pattern of a crystalline substance. When the halo is broad, it is often difficult to distinguish between a material that is truly amorphous (e.g., a true glass) and one that is merely microcrystalline. This situation exists because when microcrystallites have diameters less than about 50 Å in diameter, a similar "halo" effect is observed.

While crystalline solids offer the advantages of chemical and thermodynamic stability, amorphous solids are occasionally preferred because they undergo dissolution at a faster rate. Rapid dissolution is desirable in the case of solids, which must be dissolved prior to paren-

teral administration. Faster dissolution is also important for poorly soluble compounds administered orally, since there is often a correlation between dissolution rate and bioavailability. In fact, there are instances in which only the amorphous form has adequate bioavailability.

Amorphous solids can be precipitated from solution or obtained from melts of compounds by carrying out the solidification in such a way as to avoid the thermodynamically preferred crystallization process. They also can be prepared by disrupting an existing crystal structure. Excess free energy and entropy are incorporated into solids as they are converted into the amorphous state, since solidification occurs without permitting the molecules to reach their lowest energy states.

A. Solidification of the Melt

Amorphous solids are often created by rapidly cooling a liquid so that crystallization nuclei can neither be created nor grow sufficiently, whereupon the liquid then remains in the fluid state well below the normal freezing point. In principle, a liquid should freeze (crystallize) when cooled to a temperature below its freezing point. However, if the rate of cooling is high relative to the rate of crystallization, then the liquid state can persist well below the normal freezing point. As cooling continues there is a rise in the rate of increase of the viscosity of the supercooled liquid per unit drop in temperature. The initially mobile fluid turns into a syrup, then into a viscoelastic state, and finally into a brittle glass. A glass is, therefore, a supercooled liquid, and is characterized by an extremely high viscosity (typically of the order of 1014 Pa · s). Mechanically, if not structurally, glasses can be regarded as solids.

The characteristic temperature below which melted solids must be cooled to form a glass is the glass transition temperature T_g. The glass transition is a dynamic event that occurs at a temperature below which coordinated molecular motion becomes so slow that a liquid can be considered to take on the properties of a solid. While the exact value of this transition temperature depends on the heating rate, the glass transition temperature is generally found to be about two-thirds that of the melting temperature T_m. Glass transition temperatures reported for pharmaceuticals also follow this general rule, as can be seen in the

listing of ten pharmaceuticals that form glasses (Table 3). It is often found that the presence of impurities that facilitate glass formation increases the ratio T_g/T_m either by raising T_g or by lowering T_m. Hence one might wonder if some of the high values in the last column of Table 3 are due to partial decomposition of the drug substance upon melting. Of course, this is an important concern when employing the melt solidification procedure for the preparation of amorphous materials.

There are many examples given in the monograph *Thermomicroscopy in the Analysis of Pharmaceuticals* [9] of other compounds that solidify on the microscope hot stage to form glasses. However, Table 4 contains examples from the literature in which solidification from the melt (either by slow cooling to room temperature or by quench cooling with liquid nitrogen) has been employed as the specific method for obtaining amorphous material.

B. Reduction of Particle Size

Reduction of the particle size of crystalline materials to the microcrystalline level can yield a material incapable of exhibiting an x-ray pow-

Table 3 Pharmaceuticals Forming Glasses above Room Temperature

Compound	T_g(K)	T_m(K)	T_g/T_m
Cholecalciferol	296	352	0.84
Sulfisoxazole	306	460	0.67
Stilbestrol	308	439	0.70
Phenobarbital	321	443	0.72
Quinidine	326	445	0.73
Salicin	333	466	0.71
Sulfathiazole	334	471	0.71
Sulfadimethoxine	339	465	0.73
Dehydrocholic acid	348	502	0.69
17-β-Estradiol	354	445	0.80

Source: Ref. 84.

Table 4 Amorphous Pharmaceuticals Obtained by Solidification from the Melt

Compound	Method used	Reference
Phenylbutazone	Solidification from the melt	[85]
Indomethacin	Quench cooling using liquid nitrogen or slow cooling from the melt over 30 min	[86,87]
Felodipine	Cooling of the melt in liquid nitrogen or at ambient temperature	[88,89]
Nifedipine	Melting at 180°C followed by immersion in liquid nitrogen	[90]
Benperidol	Melt in an oven at 277°C then cool to room temperature	[91]
Acetaminophen	Solidification of the melt at −5°C/min	[92]
Sulfapyridine	Melting any crystalline form and slowly cooling the melt	[93]
Lovostatin	Melting under nitrogen, rapid cooling to 20°C below the glass transition point	[94]

der diffraction pattern. Dialer and Kuessner [95] found that when sucrose was milled in a vibratory ball mill, the ordered crystal was transformed into a glass-like structure. The increase in surface energy of milled sucrose, as measured by heat of solution, could not be accounted for by an increase in surface area alone. Hence milling disrupts the crystal lattice and imparts the excess free energy and entropy associated with amorphous substances.

Particle size reduction can be achieved using a variety of methods. Sometimes it is helpful to carry out the particle size reduction at reduced temperatures, such as at 4°C or at liquid nitrogen temperature, −196°C. In other instances, grinding with an excipient has been employed as a means of obtaining amorphous materials. Cyclodextrins and microcrystalline cellulose have been used for this purpose. It is also possible that the use of polymeric excipients may inhibit crystal growth when the amorphous solid is dissolved in water. Table 5 contains a list of compounds that have been obtained in amorphous, or partly amorphous, form by milling.

Table 5 Amorphous Pharmaceuticals Obtained by Milling

Compound	Method used	Reference
Cimetidine	Milling	[96]
FR76505	Grinding in a ball mill	[97]
Cephalexin	Grinding in an agate centrifugal ball mill for 4 hours	[98]
Indomethacin	Grinding for 4 hours at 4°C in a centrifugal ball mill; grinding the γ-form at 4°C	[57,99]
(E)-6-(3,4-Dimethoxyphenyl)-1-ethyl-4-mesitylimino-3-methyl-3,4-dihydro-2(1H)-pyrimidinone	Grinding in a stainless steel shaker ball mill for 60 minutes	[100]
9,3″-Diacetyl-midecamycin	Mixed grinding with polyvinylpyrrolidone or polyvinylpyrrolidone + hydroxypropylmethylcellulose for 9 hours	[101]
Chloramphenicol stearate	Milling in a Pulverisette 5 grinder (Fritsch) (agate mortar and balls) with colloidal silica or microcrystalline cellulose	[102,103]
Calcium gluceptate	Milling in a Pulverisette 2 grinder (Fritsch) (agate mortar and balls) for 4 hours	[104]
Chloramphenicol palmitate	Milling in a Pulverisette 0 grinder (Fritsch) (agate mortar and balls) for 85 hours	[105]
Aspirin	Grinding with adsorbents under reduced pressure	[106]
	Grinding with β-cyclodextrin	[107]
Ibuprofen	Roll mixing with β-cyclodextrin	[108]
Hydrocortisone acetate	Grinding with crystalline cellulose	[109]

Table 5 Continued

Compound	Method used	Reference
Digoxin	Milling in a Glen Creston Model M270 ball mill for 8 hours	[110]
	Comminution of 1 g at 196°C for 15 minutes in a freezer mill	[111]
Amobarbital	Ball-milling with methylcellulose, microcrystalline cellulose, or dextran 2000	[112,113]
Acetaminophen	Ball milling for 24 hours with α- and β-cyclodextrin	[114]
6-Methyleneandrosta-1,4-diene-3,17-dione	Co-grinding with β-cyclodextrin for 2 hours	[115]

C. Spray-Drying

In the pharmaceutical industry, spray-drying is used to dry heat-sensitive pharmaceuticals, to change the physical form of materials for use in tablet and capsule manufacture, and to encapsulate solid and liquid particles. This methodology is also used extensively in the processing of foods [116]. In the spray-drying process, a liquid feed stream is first atomized for maximal air spray contact. The particles are then dried in the airstream in seconds owing to the high surface area in contact with the drying gas. Spray-drying can produce spherical particles that have good flow properties, and the process can be optimized to produce particles of a range of sizes required by the particular application. The process can be run using either aqueous or nonaqueous solutions. Examples of pharmaceuticals obtained in the form of amorphous powders by spray-drying are found in Table 6.

D. Lyophilization

Lyophilization (also known as freeze-drying) is a technique that is widely employed for the preparation of dry powders to be reconstituted at the time of administration. It is a particularly useful technique in the

Table 6 Amorphous Pharmaceuticals Obtained by Spray-Drying

Compound	Method used	Reference
YM022	Spray-drying a methanol solution	[117]
α-Lactose monohydrate	Spray-drying in a Buchi 190	[118]
	Spray-drying a solution or suspension	[119]
4''-O-(4-methoxy-phenyl) acetyltylosin	Spray drying a dichloromethane solution	[120]
Salbutamol sulfate	Spray-drying of an aqueous solution in Buchi 90 spray dryer	[121]
Lactose	Spray-drying an aqueous solution	[118,122]
Furosemide	Spray-drying from a 4:1 chloroform: methanol solution at 50 and 150°C inlet temperature	[123,124]
Digoxin	Spray-drying an aqueous solution containing hydroxypropyl methylcellulose	[125]
Cefazolin sodium	Spray-drying from a 25% aqueous solution with an inlet temperature of 150°C and an outlet temperature of 100°C	[126]
9,3''-Diacetyl-midecamycin	Spray-drying of aqueous solution in the presence and absence of ethylcellulose	[127]

case of compounds that are susceptible to decomposition in the presence of moisture but that are more stable as dry solids. The physical form, chemical stability, and dissolution characteristics of lyophilized products can be influenced by the conditions of the freeze-drying cycle. In most pharmaceutical applications, lyophilization is performed on aqueous solutions containing bulking agents, and these are often chosen so as to form a coherent cake after completion of the freeze-drying process. However, lyophilization also can be employed to convert crystalline materials into their amorphous counterparts. The lyophilization process usually consists of the three stages of freezing, primary drying,

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Generation of Polymorphs

and secondary drying. For the preparation of amorphous materials, rapid freezing is employed so as to avoid the crystallization process. Both aqueous solutions and solutions containing organic solvents have been lyophilized. The primary drying phase involves sublimation of frozen water or vaporization of another solvent. This step is carried out by reducing the pressure in the chamber and supplying heat to the product. The secondary drying phase consists of the desorption of moisture (or residual solvent) from the solid.

Recently, excipients of various types have been employed in frozen solutions so as to inhibit crystallization. Cyclodextrins appear to be particularly useful for this purpose, although it is generally necessary to employ rapid freezing to liquid nitrogen temperatures to ensure that the freeze-dried product is noncrystalline. When α-cyclodextrin, which has a larger cavity than does β-cyclodextrin, is frozen at a relatively slow rate, it will cocrystallize with compounds such as benzoic acid, salicylic acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, and methyl p-hydroxybenzoate [128]. However, rapid freezing of a methyl p-hydroxybenzoate solution containing α-cyclodextrin at a benzoate/cyclodextrin ratio of 0.33 yields an amorphous solid after freeze-drying [29].

β-Cyclodextrin and its derivatives have been shown to form amorphous lyophilized products with a number of compounds, principally nonsteroidal antiinflammatory agents. Examples from the literature of excipients and pharmaceuticals prepared as amorphous materials by lyophilization are given in Table 7.

E.   Removal of Solvent from a Solvate or Hydrate

Solids can sometimes be rendered amorphous by the simple expedient of allowing solvent molecules of crystallization to evaporate at modest temperatures. If the solvent merely occupies channels in the crystal structure, the structure often remains intact, but when the solvent is strongly bonded to molecules of the host, the structure frequently will collapse when the solvent is removed and one obtains an amorphous powder. A few examples of amorphous solids obtained in this manner are found in Table 8.

Table 7 Amorphous Pharmaceuticals Obtained by Lyophilization

Compound	Method used	Reference
Lactose	Lyophilization of a 5% Aqueous Solution	[130]
MK-0591	Lyophilization	[131]
Raffinose	Lyophilization of a 10% aqueous solution frozen at −45°C	[132]
Sucrose	Lyophilization of 10% aqueous solutions	[133]
Dirithromycin	Freeze-drying from methylene chloride solution	[134]
Cefalexin	Aqueous solution frozen at −196°C, then freeze-dried	[135]
	Lyophilization of a saturated aqueous solution	[136]
Calcium gluceptate	Freeze-drying from 2% aqueous solution	[137]
Griseofulvin	Freeze-drying of solutions of griseofulvin or of solutions of mixtures of griseofulvin and mannitol in dioxane or 1:1 dioxane-water with fast freezing in liquid nitrogen	[138]
Tolobuterol hydrochloride	Freeze-drying of aqueous solution	[139]
E1040	Freeze-drying of aqueous solution	[140]
Glutathione	Freeze-drying of a 5% aqueous solution	[141]
Aspirin	Freeze drying of an aqueous solution in the presence of 1.0% hydroxypropyl-β-cyclodextrin	[142]
Ketoprofen	Freeze-drying in the presence of heptakis-(2,6-O-dimethyl)-β-cyclodextrin	[143]
	Freeze-drying with β-cyclodextrin (rapid freezing with liquid nitrogen)	[144]
Glibenclamide	Freezing at liquid nitrogen temperature, freeze-drying over 24 hours	[145]

Table 7 Continued

Compound	Method used	Reference
Naproxen	Colyophilization (223K and 0.013 torr) of naproxen and hydroxyethyl-β-cyclodextrin, or hydroxypropyl-β-cyclodextrin	[146]
Sodium ethacrynate	Rapid freezing of an aqueous solution to −50°C, followed by freeze-drying	[147]
p-Aminosalicylic acid	Colyophilization of p-amino-salicylic acid in aqueous solution with pullulan	[148]
Ceftazidime	Freeze-drying a nearly saturated aqueous solution of the free acid	[149]
Cefaclor	Freeze-drying from a nearly saturated aqueous solution	[149]
Cephalothin sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefamandol sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefazolin sodium	Freeze-drying an aqueous solution at low temperature	[149]
Nicotinic acid	Freeze-drying in the presence of β-cyclodextrin (fast-freezing); and heptakis (2,6-O-dimethyl)-β-cyclodextrin	[150]

F. Precipitation of Acids or Bases by Change in pH

If the level of supersaturation is carefully controlled, it is often possible to avoid crystallization when a water-soluble salt of a weak acid is precipitated with a base, or when a water-soluble salt of a weak base is precipitated with an acid. When crystalline iopanoic acid is dissolved in 0.1 N NaOH, and 0.1 N HCl is added, an amorphous powder is precipitated [43]. A similar phenomenon is observed in the case of the precipitation of piretanide [155]. Another example in this genre is the

Table 8 Amorphous Pharmaceuticals Obtained by Solvent Removal

Compound	Method used	Reference
Tranilast anhydrate	Dehydration of the monohydrate over P₂O₅	[151]
Raffinose	Lyophilization and heat drying of the pentahydrate	[192]
Erythromycin	Heating the dihydrate for 2 hours at 135°C in an oven, and then cooling to room temperature	[152,53]
Calcium DL-pantothenate	Drying the methanol:water 4:1 solvate in vacuo at 50–80°C	[154]

precipitation of amorphous calcium carbonate, which occurs when a calcium chloride solution is combined with a sodium carbonate solution at 283K [156].

G. Miscellaneous Methods

Earlier during the discussion on the preparation of polymorphs, the doping of crystals was mentioned as a technique for encouraging the formation of one type of polymorph over another. Similarly, if a dopant is employed at levels that will disrupt the crystal lattice, the substance can be made to solidify as an amorphous material. Duddu and Grant [157] observed changes in the enthalpy of fusion of (−)-ephedrinium 2-naphthatenesulfonate when the opposite enantiomer, (+)-ephedrinium 2-naphthalenesulfonate, was added as a dopant.

When m-cresol was added to a suspension of insulinotropin crystals grown from a normal saline solution, the crystals were immediately rendered amorphous. It was postulated [158] that the m-cresol molecules diffused into the crystals through solvent channels and disturbed the lattice interactions that ordinarily maintained the integrity of the crystal. When zinc acetate or zinc chloride was added to the suspension, the zinc ion stabilized the crystal lattice so that the subsequent addition of m-cresol did not alter the integrity of the crystals.

Sometimes solvents exert a similar effect. When a small amount of ethyl acetate is added to a calcium chloride solution prior to addition

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of sodium fenoprofen, the calcium fenoprofen that precipitates has a low degree of crystallinity [159]. Similarly, when calcium DL-pantothenate is precipitated from methanol or ethanol solution by the addition of acetone, ether, ethyl acetate, or other solvents, the precipitate obtained is found to be amorphous [154].

V. SUMMARY

The pharmaceutical development scientist who is assigned the task of demonstrating that a substance exhibits only one crystalline form, or that of discovering whether additional forms exist, can utilize the techniques outlined in this chapter as a starting point. Upon completion of this program, one can certainly conclude that due diligence has been employed to isolate and characterize the various solid-state forms of any new chemical entity. One should always be aware that nuclei capable of initiating the crystallization of previously undiscovered forms might be lurking around the laboratory, ready to confound the investigator should their effects become known. In addition, the phenomenon of "disappearing polymorphs" can come into play, and techniques that formerly yielded the same crystals every time may subsequently yield crystals of another, more stable form. In the future, the use of computer simulations of alternative crystallographic structures will suggest how much laboratory work might be required to isolate the polymorphs or solvates of a given compound. Until then, the empirical approach remains superior.

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Methods for the Characterization of Polymorphs and Solvates

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